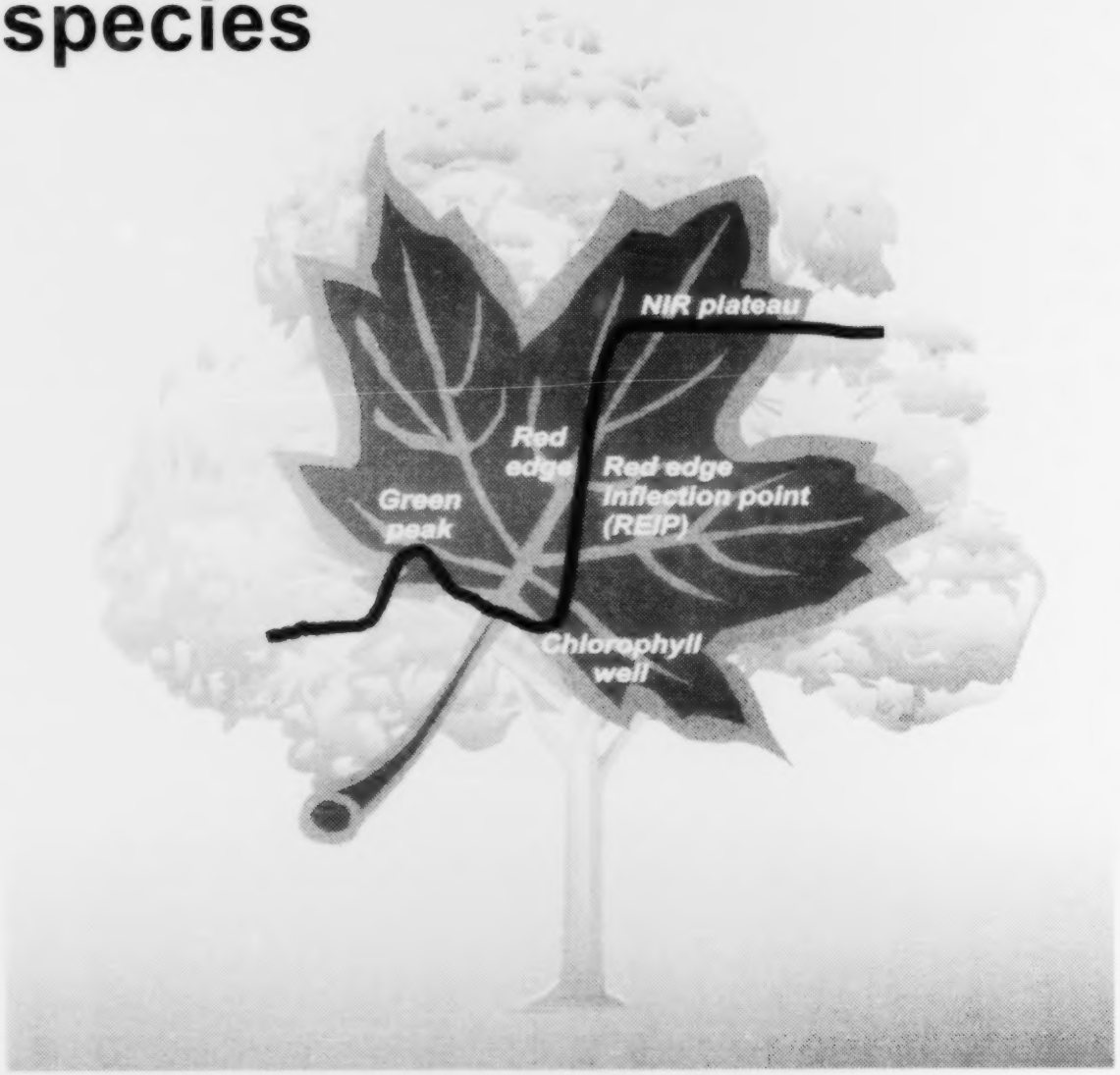
Forest Research Report No. 156

Natural and stress-induced effects on leaf spectral reflectance in Ontario species



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Natural and stress-induced effects on leaf spectral reflectance in Ontario species

by

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Summary

This study explores some of the biological factors influencing leaf-based spectral reflectance, and endeavours to answer the questions: (1) Can Ontario plant species be satisfactorily distinguished by their leaf spectra? (2) Can spectral properties be used to identify physiological strain in plants before visual symptoms appear? and (3) How strongly do factors other than species and stress affect spectral properties? Forty-four species were examined for species effects. Some general patterns were revealed, including: low reflectance in the near infrared region for conifers compared to broadleaved species; higher reflectance in the blue wavebands for species with blue hues in their foliage; and more rapid decay of the green spectral peak in deciduous tree and shrub species. However, the influence of species was easily superceded by other factors, such as leaf age, leaf side, and stress status. The effects of stresses such as senescence and herbicide on leaf reflectance were evident prior to the appearance of visual symptoms such as chlorosis, browning, and reduced growth. The application of spectral indices was a useful means to quantify previsual changes in spectral reflectance. The red edge inflection point was well correlated with chlorophyll content, confirming previous studies. Pooling a range of coniferous species and stock types, the Photochemical Reflectance Index (PRI) was a promising early indicator of strain which may have broad application; within species, additional indices were useful. Normalized difference vegetation indices (NDVI) had limited correlation to pigments and other physiological indicators of stress status. Altogether, 11 indices reported in the literature were tested. These findings suggest that leaf reflectance may be an effective early indicator of physiological strain, and underpin our efforts to develop monitoring tools at remote scales to aid sustainable forest management.

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Contents

Introduction **1**

Methods **2**

Experiments 2

Experiment 1: Species effects 2
Experiment 2: Adaxial versus abaxial foliar effects 2
Experiment 3: Sun versus shade effects 2
Experiment 4: Leaf age effects 2
Experiment 5: Tree age effects 2
Experiment 6: Diurnal effects 2
Experiment 7: Senescence stress 3
Experiment 8: Herbicide and girdling stresses 4

Techniques 4

Spectral reflectance 4
Chlorophyll fluorescence 5
Chlorophyll *a* and *b* and total carotenoids 6
Photosynthesis, stomatal conductance, and intercellular CO_2 7

Results **7**

Species effects 7
Adaxial versus abaxial effects 7
Sun versus shade effects 14
Leaf age effects 14
Tree age effects 18
Diurnal effects 18
Senescence stress effects 18
Herbicide and girdling stress effects 18

Discussion **25**

Factors affecting nonvisual leaf reflectance 25

Chemical constituents 25
Leaf structure 26
Leaf age 27

Species differences 28
Stress effects 28

Conclusions **30**

References **31**

List of Figures

Figure 1. Typical spectral reflectance curve for green foliage. 7
Figure 2. Leaf spectral reflectance of Ontario species. A. Evergreen tree/shrub species. B. Deciduous tree/shrub species. C. Herbaceous/moss/lichen species. D. Wetland/aquatic species. 8
Figure 3. Abaxial and adaxial leaf reflectance. 12
Figure 4. Sun and shade leaf reflectance 15
Figure 5. Leaf age effects on reflectance. 15
Figure 6. Tree age effects on reflectance. 16
Figure 7. Diurnal effects on reflectance. 16
Figure 8. Senescence effects on reflectance of white pine. Chlorophyll *a* concentrations in µg/g DW are shown. 17
Figure 9. Senescence effects on reflectance of sugar maple. Chlorophyll *a* concentrations in µg/cm^2 and leaf colour are shown. 17
Figure 10. Reflectance of shade-grown or transferred (shade to sun) seedlings of sugar maple. 19
Figure 11. Reflectance of conifer species subjected to herbicide and girdling treatments. 19

List of Tables

Table 1. Species used in the spectral database. 3
Table 2. Spectral indices and features. 5
Table 3. Spectral index values for leaf spectra of 44 Ontario plant species. 13
Table 4. Spectral index values for abaxial and adaxial leaf surfaces. 14
Table 5. Spectral index values for sun and shade leaves of *Acer succharum* 20
Table 6. Spectral index values for leaves of different age, size, or type. 20
Table 7. Spectral index values for trees of different age 20
Table 8. Spectral index values of *Acer saccharum* at different times of the day. 21
Table 9. Spectral index values of *Pinus strobus* with different concentrations of chlorophyll. 21
Table 10. Spectral index values of *Acer saccharum* with different concentrations of chlorophyll and various stages of autumn coloration. 21
Table 11. Spectral indices, pigment concentrations, chlorophyll fluorescence, and photosynthesis in *Acer saccharum* under artificially induced senescence in late summer. 22
Table 12. Spectral indices, pigment concentrations, chlorophyll fluorescence (CF), stem growth and percent browning of *Pinus banksiana* exposed to herbicide or girdling stress. 23
Table 13. Spectral indices, pigment concentrations, chlorophyll fluorescence (CF), stem growth and percent browning of *Picea mariana* exposed to herbicide or girdling stress. 23
Table 14. Spectral indices, pigment concentrations, chlorophyll fluorescence (CF), stem growth and percent browning of *Pinus resinosa* exposed to herbicide or girdling stress. 24
Table 15. Correlation between spectral indices, pigment concentrations, and chlorophyll fluorescence (CF), vs. stem growth and percent browning in conifers exposed to herbicide or girdling stress. 24

Introduction

Light contacting the leaf surfaces of plants is either absorbed, transmitted, or reflected. The pattern of reflected light – or **spectral reflectance** – in leaves is quite distinct from that of soil and other materials, and is the product of leaf chemical, physical, and physiological characteristics (Zarco-Tejada et al. submitted *a, b*; Treitz and Howarth 1999; Carter 1998; Dendron Resource Surveys 1997; Middleton et al. 1998; Gitelson and Merzlyak 1996; Treitz and Howarth 1996; Gastellu-Etchegorry et al. 1995; Carter 1991; Miller et al. 1991; Clark and Lister 1975). These features include *foliar anatomy* such as, leaf thickness, epicuticular wax qualities, and mesophyll structure; *pigments and other biochemical contents* such as chlorophylls, carotenoids, anthocyanins, phenolics, proteins, lignin, and cellulose; and *physiological status* such as water content, photosynthetic efficiency, and fluorescence from chlorophyll and other molecules.

Spectral reflectance has been the subject of extensive study at both the leaf level and in remote sensing (Carter et al. 1998, Dendron Resource Surveys 1997, Knapp and Carter 1998, Zwiggelaar 1998, Gong et al. 1997, Rock et al. 1989). In forestry, remote sensing efforts have been directed to the possible use of reflectance as a surrogate for ground-based identification of species, biomass production, pest infestation, and forest decline. In particular, considerable interest exists in modern hyperspectral techniques that provide high spectral and spatial resolution (Dendron Resource Surveys 1997, Martin and Aber 1997, Treitz and Howarth 1996). At the leaf level, deriving a basic understanding of biophysical controls on spectral properties has been of great interest, and is the foundation for the development of remote sensing sampling strategies and data interpretation methods.

In view of the many biological, atmospheric, and instrumental influences on reflectance, the challenges posed by remote sensing are considerable. Although many of the technological challenges have been successfully overcome (Dendron Resource Surveys 1997), much remains to be understood about the biology of spectral reflectance. Specifically, the fundamental processes affecting spectral properties at the leaf level are not yet fully understood (Treitz and Howarth 1996).

Leaf-based spectral reflectance is the subject of this study. In particular, we are interested in the following questions:

- Can Ontario plant species be satisfactorily distinguished by their leaf spectra?
- Can spectral properties be used to identify physiological strain in plants before visual symptoms appear?
- How strongly do factors other than species and stress affect spectral properties of Ontario plants?

These questions are relevant to a current effort to identify bioindicators of forest condition in Ontario (Mohammed et al. 1997), which is focussed on developing early indicators of physiological strain in support of sustainable forest management.

Methods

This section describes the experiments conducted to address the questions of interest followed by a summary of techniques.

Experiments

Experiment 1: Species effects

Forty-four species were included in this study, providing examples of evergreen tree/shrub, deciduous tree/shrub, herbaceous/moss/lichen, or wetland/aquatic plants (Table 1). Specimens were from natural or planted areas in the Algoma region of central Ontario and were chosen for good overall health as evidenced by colour and lack of obvious damage or pest infestation. All collections were made during the period of July-August 1997, between 10 am and 4 pm. Five fully developed current-year leaves or needles were collected from mature plants or trees and stored in ziploc plastic bags in a 5°C cooler until spectra could be done at the laboratory. Spectra were recorded (see ***Techniques***) at room temperature, from adaxial (upper) leaf surfaces.

Experiment 2: Adaxial versus abaxial foliar effects

Species studied were *Acer rubrum*, *A. saccharinum*, *A. saccharum*, *Fraxinus americana*, *Picea mariana*, *Pinus banksiana*, *P. strobus*, and *Tsuga canadensis*. The identification of foliar surfaces as either abaxial or adaxial was based on anatomical features (Esau 1977). For broadleaves, the adaxial surface is adjacent to the palisade mesophyll layer, while the abaxial surface is adjacent to the spongy mesophyll layer. For conifers, the adaxial surface is the flat (or less curved) side closest to the needle xylem, and the abaxial surface is usually the more curved side closest to the phloem. To aid designation of conifer needles, the adaxial surface of conifer needles is directed toward the axis of needle extension (and is typically the deepest green of all sides, with fewer stomata than other facets), while the abaxial side is directed away from the axis. Fully developed foliage from 5 healthy, mature donors were collected and stored briefly in ziploc plastic bags in a 5°C cooler for spectral analysis.

Experiment 3: Sun versus shade effects

Sun and shade leaves from 5 mature trees of *A. saccharum* were collected and stored in ziploc plastic bags in a 5°C cooler, and leaf spectra of their adaxial surfaces were recorded.

Experiment 4: Leaf age effects

Mature *A. saccharum*, *Pinus strobus*, and *Tsuga canadensis* were used to provide samples of mature, intermediate, and immature current-year foliage in maple and hemlock, and current and 1-year-old needles of pine. Adaxial surfaces of leaves from 5 trees were sampled for spectral features.

Experiment 5: Tree age effects

Acer saccharum, *Picea glauca*, and *Betula papyrifera* of various ages – mature (20+ years), adolescent (12-15 years), and juvenile (2-3 years) – were used to provide fully developed foliage (current-year for *Picea*). Foliage from 5 trees per age category was collected and stored in ziploc plastic bags in a 5°C cooler and analyzed for spectral features.

Experiment 6: Diurnal effects

Potted *A. saccharum* saplings, grown outdoors under 50% shade, were used for assessment of diurnal effects. Fully developed leaves of 5 healthy plants were tagged for repeated non-destructive measurement during a sunny day (August 18, 1998), using the same

spot (indicated with a 2-cm drawn circle) on each leaf throughout the day. Plants were sampled at 0800 h, 1000 h, 1200 h, 1400 h, 1600 h, 1800 h, and 2000 h. *In situ* analysis was done of spectral reflectance and chlorophyll content (SPAD-502 Minolta Chlorophyll Meter Minolta Camera Co., Ltd., Japan). Air temperature and photosynthetic photon flux density (PPFD) were measured at each sampling time.

Experiment 7: Senescence stress

Fully developed foliage from mature trees of *Pinus strobus* (current-year foliage) and *A. saccharum* were collected September 16, 1997 from the Ontario Forest Research Institute's (OFRI) Arboretum in Sault Ste. Marie. Ten leaves or fasicles from each species were selected to represent a range of visible chlorosis in the case of *Pinus*, and a gradient in autumn coloration (from deep green to bright red) in *A. saccharum*. *In situ* spectral analysis was done, then samples were stored in ziploc bags on ice for subsequent laboratory analysis of chlorophyll *a* & *b* and total carotenoids.

In a second experiment, potted *A. saccharum* saplings growing outdoors in full sun (at OFRI) were used. On August 25, 1997, one-half (10) of the pots were moved to a nearby shaded housing frame that provided 50% shade from black shade cloth. Plants were watered and fertilized as required. On September 23, leaves were collected for analysis of spectral reflectance, F_v/F_{max}, gas exchange and pigments (chlorophyll *a*, *b*, and total carotenoids—see ***Techniques***). Gas exchange in shaded plants was analyzed in the shaded growing environment (PPFD 73.5 $\mu mol.m^{-2}.s^{-1}$), while that in transferred plants was assessed in the sun (1437 $\mu mol.m^{-2}.s^{-1}$). No changes in colour were visible to the unaided eye at the time of assessment.

Table 1. *Species used in the spectral database.*

Species	Common name
Evergreen Tree/Shrub	
Abies balsamea	balsam fir
Picea abies	Norway spruce
Picea glauca	white spruce
Picea mariana	black spruce
Pinus banksiana	jack pine
Pinus resinosa	red pine
Pinus rigida	pitch pine
Pinus strobus	eastern white pine
Thuja occidentalis	eastern white cedar
Tsuga canadensis	eastern hemlock
Deciduous Tree/Shrub	
Acer pensylvanicum	striped maple
Acer rubrum	red maple
Acer saccharinum	silver maple
Acer saccharum	sugar maple
Alnus crispa	green alder
Betula alleghaniensis	yellow birch
Betula papyrifera	paper birch
Fagus grandifolia	American beech
Fraxinus americana	white ash
Juglans nigra	black walnut
Larix laricina	tamarack
Populus deltoides	cottonwood
Populus tremuloides	trembling aspen
Quercus rubra	red oak
Rubus idaeus	red raspberry
Salix humilis	upland willow
Tilia americana	American basswood
Herbaceous/Moss/Lichen	
Aster macrophyllus	large-leaved aster
Calamagrostis canadensis	Canada blue-joint grass
Cladina rangiferina	reindeer lichen
Epilobium angustifolium	fireweed
Hypogymnia physodes	monk's hood lichen
Polytrichum juniperinum	juniper haircap moss
Pteridium aquilinum	bracken fern
Trifolium repens	white clover
Wetland/Aquatic	
Bidens cernua	nodding bur-marigold
Carex lacustris	lakeband sedge
Impatiens capensis	jewelweed
Myrica gale	sweet gale
Nymphaea odorata	fragrant white water lily
Potentilla palustris	marsh cinquefoil
Sagittaria latifolia	broad-leaved arrowhead
Spirea alba	narrow-leaved meadowsweet
Viola nephrophylla	northern bog violet

Experiment 8: Herbicide and girdling stresses

Seven-year-old *Pinus banksiana*, *P. resinosa*, *Picea mariana* and *P. glauca* from a 1992 outplant trial of OFRI's Stock Quality Assessment Program located at the OFRI Arboretum were used. A total of 8 stocklots (2 *P. banksiana*, 1 *P. resinosa*, 4 *P. mariana*, and 1 *P. glauca*) were included. In early June 1998, 4 stress treatments and a control group were established. Treatments were tested prior to this study to adversely affect growth and overall health, and possibly survival over the course of the season.

Herbicide treatments were glyphosate (83.5% active ingredient (a.i.)), imazapyr (83.5% a.i.), and triclopyr (44.4% a.i.), injected into the main stem using an EZJECT™ capsule injection system (Monsanto Co., USA) at a rate of 1 capsule per tree. Glyphosate is a non-selective herbicide that affects protein synthesis and the biosynthetic pathways leading to growth. Imazapyr inhibits protein synthesis and may disrupt photosynthate translocation, induce hormone imbalance from interruption of source/sink relationships, and interfere with DNA synthesis and cell growth. Triclopyr behaves similarly to other auxin-type herbicides and affects cell wall plasticity and nucleic acid metabolism, leading to uncontrolled cell division and growth, and vascular tissue destruction (Ahrens 1994).

For the girdling treatment, a ring of bark (and phloem) tissues 5-cm high and 2- to 3-mm thick was removed with a sharp knife from the basal part of the main stem (a few cm above ground).

At the end of July (approx. 7 weeks after treatment), assessments of spectral reflectance, F_v/F_{max}, and pigment concentrations were made on a random selection of 5 trees per treatment and stocklot. In October, percent browning and stem growth were assessed on all 367 trees.

Techniques

Spectral reflectance

Leaf spectral reflectance was examined with a portable UniSpec® Spectral Analysis System / Reflectometer (PP Systems, Haverhill, MA), interfaced to a Hewlett Packard HP 1000CX Palmtop computer. Technical specifications of the UniSpec include a wavelength range of 300 to 1150 nm, Raleigh spectral resolution of <10 nm, bin size (diode array) of 3.3 nm, and an absolute accuracy of <0.3 nm. The light source is an integral 6.8 W halogen bulb, which was allowed to warm up for 3 minutes prior to use. For conifer needles, a mini-foreoptic (0.5-mm measurement area) was used; with broadleaves, the standard foreoptic (1.5-mm measurement area) was used. Fifty scans were integrated per sample, using an integration time of 10 ms.

A single leaf or needle was inserted into either the mini clip (conifer) or standard clip (broadleaf). A dark scan (bulb shutter closed) was done, followed by a reference scan with a white Spectralon reflectance standard (Labsphere Inc., North Sutton, NH), then a foliar scan. Percent reflectance was calculated from the raw and reference data.

Reflectance data were used to calculate the spectral features or indices shown in Table 2. Spectral indices are algebraic combinations of reflectance measurements in 2 or more spectral wavebands.

The major features of a typical reflectance curve for green foliage are illustrated in Figure 1 (Rock et al. 1989). Chlorophyll and other pigments absorb electromagnetic radiation (EMR) strongly in the visible wavelengths, thus reflectance is low in these regions. Chlorophyll in particular absorbs EMR strongly in the blue (400 to 500 nm) and red (600 to 700 nm) portions of the visible spectrum, with relatively less

Table 2. *Spectral indices and features.*

Spectral index or feature	Symbol	Meaning	Reference
Visible ratios			
R440/R690	B/R	blue/red	Lichtenthaler et al. 1996b (used fluorescence ratio)
(R531-R570)/(R531+R570)	PRI	photochemical reflectance index	Peñuelas et al. (1995b)
Visible/NIR ratios			
R440/R740	B/FR	blue/far red	Lichtenthaler et al. 1996b (used fluorescence ratio)
R750/R550	FR/G	far red/green	Gitelson and Merzlyak 1996, Lichtenthaler et al. 1996a
(R800-R680)/(R800+R680)	NDVI	normalized difference vegetation index	Guyot 1990, Lichtenthaler et al. 1996a
Red edge reflectance ratios			
R700	RE	red edge	Gitelson et al. 1996
R694/R760	R/FR	red/far red	Carter et al. 1996
R750/R700	FR/R	far red/red	Gitelson and Merzlyak 1996, Lichtenthaler et al. 1996a
(R734-R747)/(R715-R726)	FR726	far red 726	Moss and Rock 1991, Vogelmann et al. 1993
(R734-R747)/(R715-R720)	FR720	far red 720	Vogelmann et al. 1993 (modified)
Derivative red edge feature			
Red edge inflection point	REIP	red edge inflection point	Miller et al. 1990

absorption in the green (500 to 600 nm) portion. This generates the so-called *green peak* and *chlorophyll well* features in the visible band. The pigments absorb little EMR in the near infrared (NIR) region of the spectrum. This combined with scattering of EMR by the internal leaf structure results in a very high reflectance in the NIR – the so-called *NIR plateau*. The rapid rise in reflectance between the *chlorophyll well* and the *NIR plateau* has been called the *red edge*.

Chlorophyll fluorescence

Foliage was dark-adapted for 30 minutes at room temperature or natural growing temperature outdoors (using metal dark-adapting clips). Dark adaptation is necessary to oxidize electron carriers in photosynthetic tissues, so that when the tissues are subsequently exposed to bright light, maximal fluorescence may be observed (Walker 1985). Chlorophyll fluorescence was analyzed with a Pulse Amplitude Modulation (PAM-2000) fluorometer (Heinz Walz GmbH, Effeltrich, Germany), an instrument that has been used widely in basic and applied fluorescence research (reviewed by Mohammed et al. 1995). The leaf or a cluster of needles was positioned in the PAM-2000 leaf clip holder, which exposes a 1-cm diameter sample area to the fibreoptic light emitter and detector array.

The ratio of variable to maximal fluorescence (F_v/F_{max}) was determined for the adaxial (upper) leaf surface. F_v/F_{max} quantifies the maximal efficiency of photon capture by open photosystem II (PSII) reaction centres (Butler and Kitajima 1975), and is one of the most widely used chlorophyll fluorescence features (Mohammed et al. 1995). It is calculated as $F_v/F_{max} = (F_{max} - F_o)/F_{max}$ where F_{max} is the maximal fluorescence yield

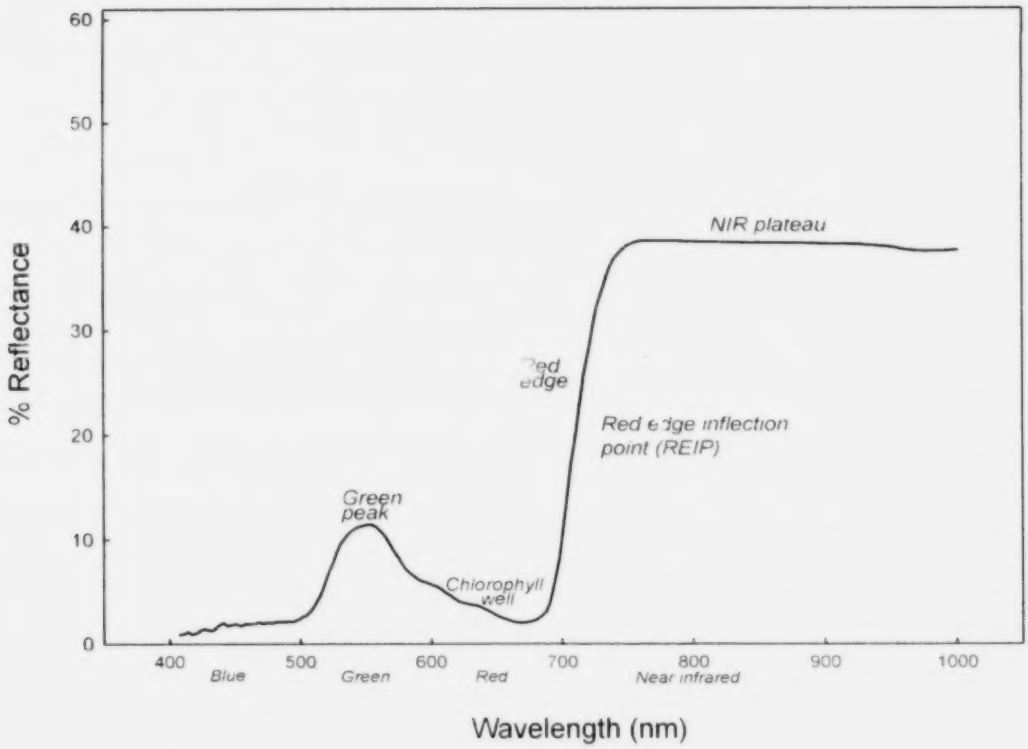

Figure 1. *Typical spectral reflectance curve for green foliage.*

of a dark-adapted sample, with all PSII reaction centres fully closed, and F_o is the minimum fluorescence yield of a dark-adapted sample, with all PSII reaction centres fully open (Van Kooten and Snel 1990). F_o is measured first, using a red measuring light with a maximum emission of 655 nm, at a very low PPFD of about 0.1 $\mu mol.m^{-2}.s^{-1}$, and a modulation frequency of 600 Hz. F_{max} was determined by exposing the sample to a saturating pulse of light (> 6000 $\mu mol.m^{-2}.s^{-1}$, < 710 nm wavelength) of 0.8 s duration and 20 kHz modulation frequency. Fluorescence from the plant is detected at wavelengths > 700 nm. The F_v / F_{max} was sampled using an automated procedure of the PAM-2000, and standard methodologies (Heinz Walz GmbH 1993).

Chlorophyll *a* and *b* and total carotenoids

Foliage was collected and stored at –23°C until analysis. For conifers, 2 to 4 g (fresh weight) of foliage was weighed out and ground in liquid N_2 with 0.2 g used for pigment extraction and the remainder used for dry weight determination. For *A. saccharum*, 2 2.3-cm circles were cut out of the leaf. One circle was ground in liquid N_2, weighed, and placed in a 15-ml centrifuge tube. The second circle was weighed, oven dried at 80°C for 24 hours, and reweighed. Ten ml of N,N-dimethylformamide (Spectralanalyzed grade, Fisher) were added to the tube. Tubes were placed horizontally in a darkened 4°C orbital shaker set to 100 rpm for 2 hours to extract pigments. Tubes were centrifuged at 5°C and 5,000 g for 20 minutes. Tubes were placed in a dark, 4°C refrigerator for 20 minutes, then removed and 3

ml of supernatant placed in a cuvette and the absorbance measured at 663.8 nm, 646 nm, and 480 nm with a Cary 1 spectrophotometer. Chlorophyll *a*, chlorophyll *b*, and total carotenoid concentrations were determined using the extinction coefficients derived by Wellburn (1994).

Photosynthesis, stomatal conductance, and intercellular CO_2

Leaf gas exchange was measured using a LI-6200 portable photosynthesis system (LI-COR, Lincoln, Nebraska) and 0.25 l cuvette. Net photosynthesis (P_n, µmol $CO_2.m^{-2}.s^{-1}$), leaf stomatal conductance to water vapour (COND, $mol.m^{-2}.s^{-1}$) and intercellular CO_2 concentration (CINT, ppm) were estimated from values averaged for 2 consecutive 10 s determinations. Gas exchange features were represented on the basis of 1-sided leaf area.

Results

Species effects

All species followed a generalized pattern of spectral response in which reflectance was lower in the blue and red wavebands, and highest in the NIR and green regions (Figure 2). The species differed primarily according to broad taxonomic designation, with the following patterns of response:

- Evergreen or coniferous species had relatively low reflectance in the NIR region, generally not exceeding 40% reflectance, compared to 50% or greater reflectance in broadleaved species. *Picea* spp. had the lowest NIR reflectance.
- Species with blue hues in their foliage produced relatively higher reflectance in the blue wavebands from 400 to 500 nm. These were *Abies balsamea*, *Calamagrostis canadensis*, *Cladina rangiferina*, and *Hypogymnia physodes*. Also, in the latter 3 species, the visible bands, but not the NIR, of the spectrum were elevated.
- Deciduous tree and shrub species sampled here frequently had more rapid decay of the green peak.

Spectral indices for the 44 species are shown in Table 3. The characteristics discussed above are quantified in indices such as FR/G, which was low in evergreen or coniferous species as a result of low NIR. Conversely, R/FR was high in these species. Indices from the blue region were highest in *A. balsamea*, *P. mariana*, and *T. canadensis*, and were relatively high in those species with blue-tinged foliage such as the lichens and *Calamagrostis canadensis*. Most indices had a substantial range between the minimum and maximum values, with maximum values as much as 5 times the minimum (B/FR). Smaller ranges were noted for REIP, photochemical reflectance index (PRI), and B/R.

Adaxial versus abaxial effects

Abaxial foliar surfaces generally produced higher reflectance in the visible (VIS) wavebands than adaxial surfaces (Figure 3). This effect was more pronounced in broadleaved species compared to conifers, and in species whose foliage had clear colour differences between the abaxial and adaxial sides, e.g., *Acer rubrum* and *A. saccharinum*. In *Pinus banksiana* and *Picea mariana*, abaxial and adaxial surfaces produced very similar spectra.

Indices involving the VIS region, particularly the red and green wavebands (FR/G, R/FR, FR/R), clearly differentiated abaxial from adaxial surfaces in the broadleaved species (Table 4). Both FR/G and FR/R were lower (by as much as 2.6-fold) and R/FR was higher (up to 3-fold) in abaxial surfaces.

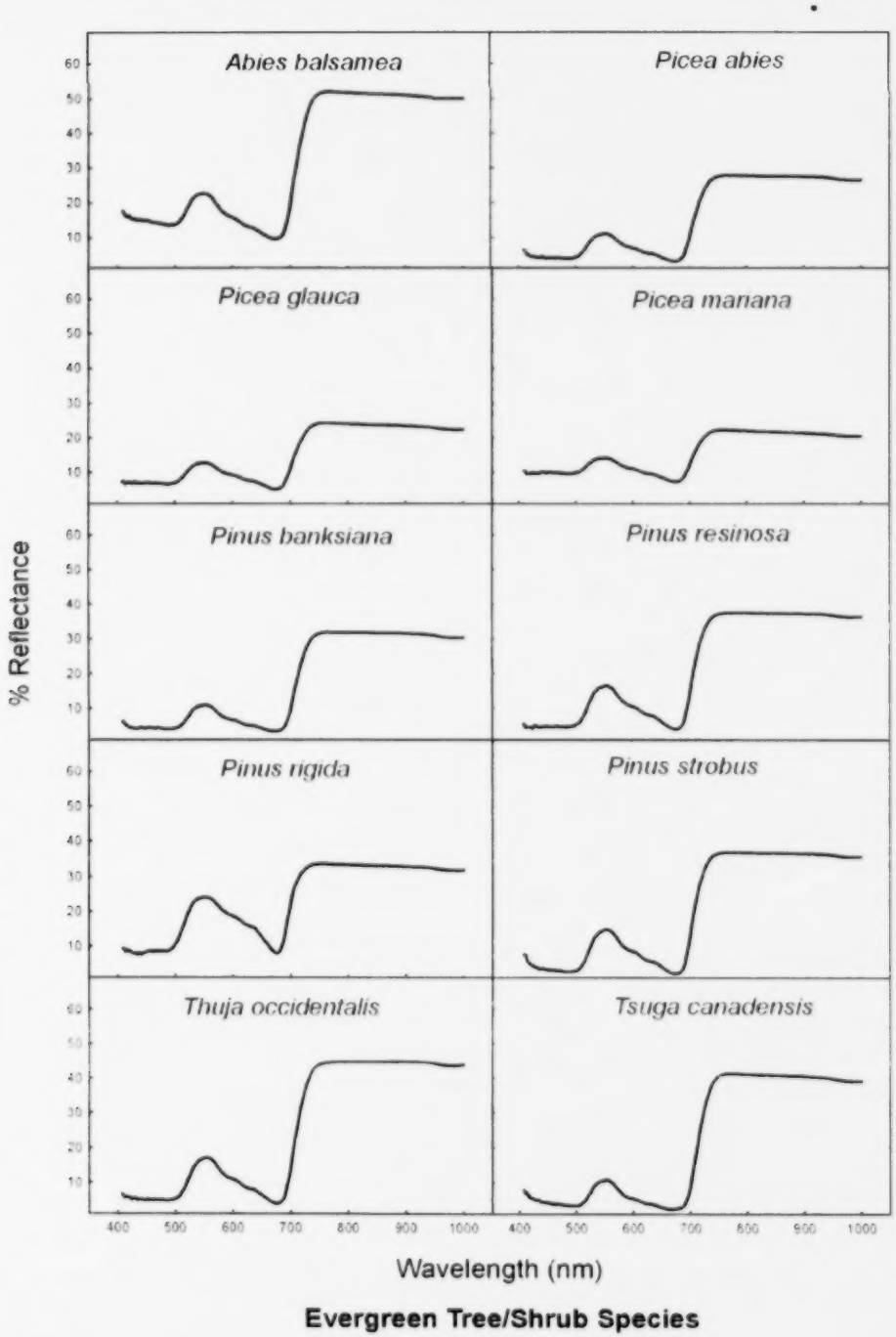

Figure 2. *Leaf spectral reflectance of Ontario species. A. Evergreen tree/shrub species.*

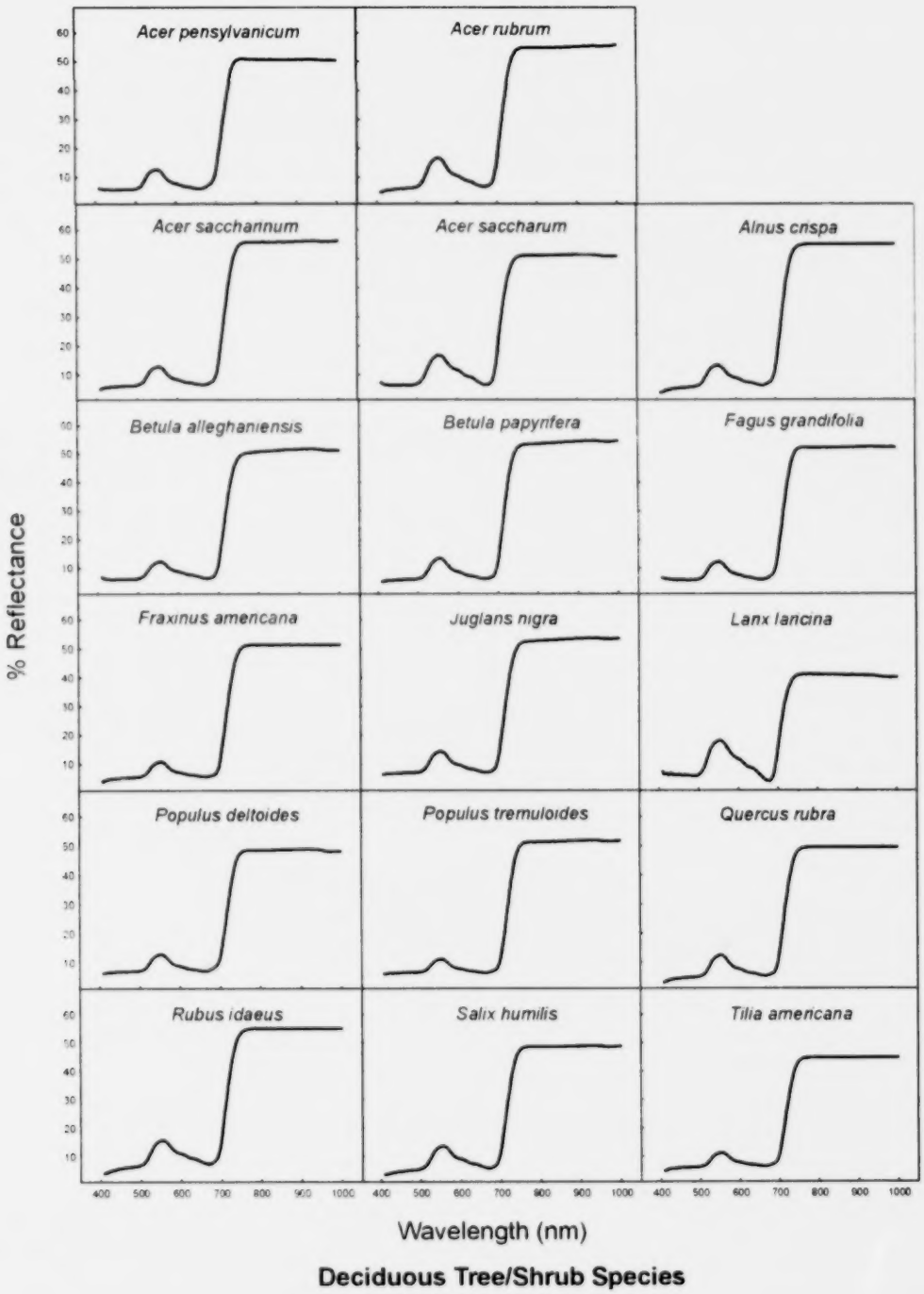

Figure 2 (*con't*). *Leaf spectral reflectance of Ontario species. B. Deciduous tree/shrub species.*

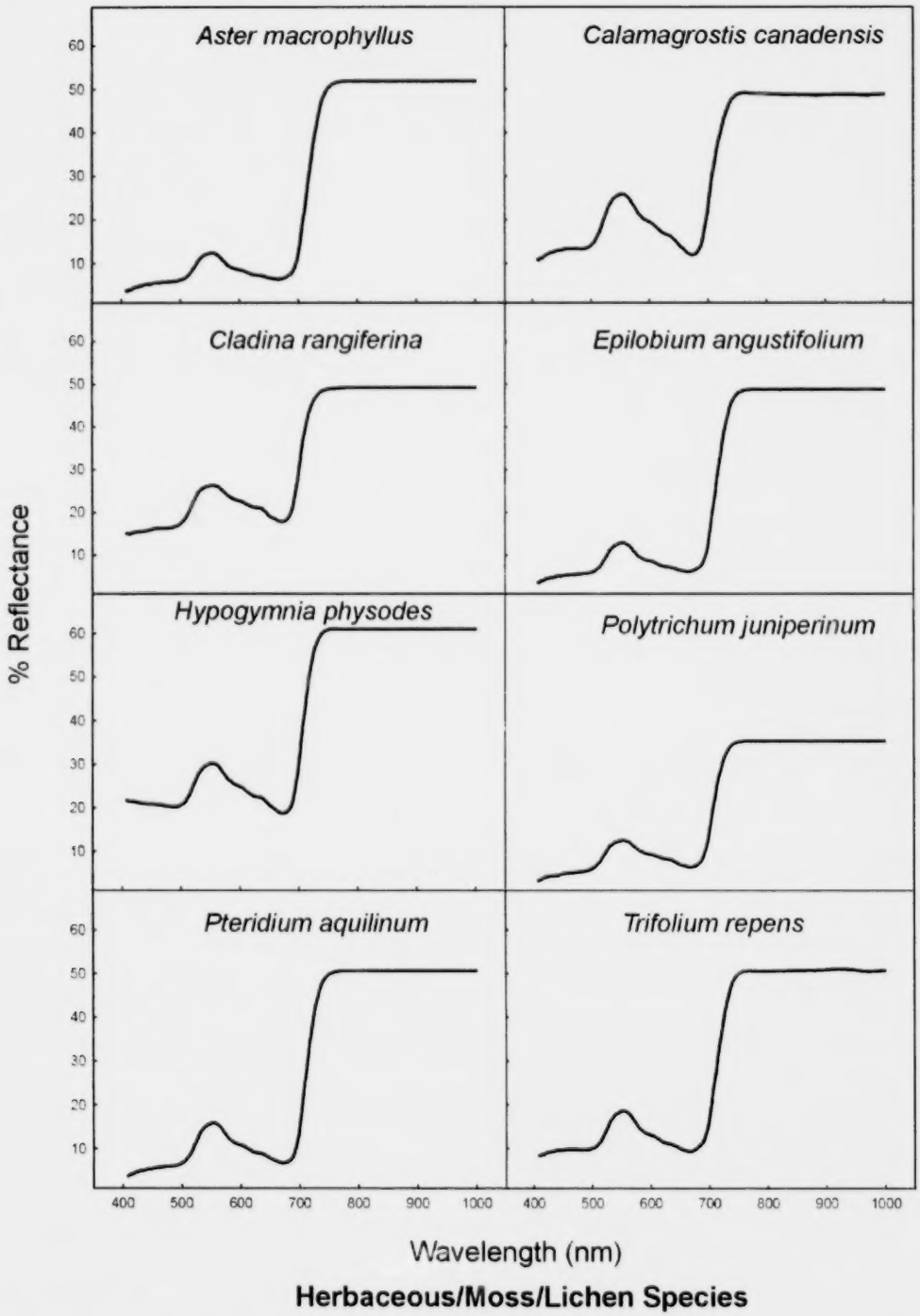

Figure 2 (*con't*). *Leaf spectral reflectance of Ontario species. C. Herbaceous/moss/lichen species.*

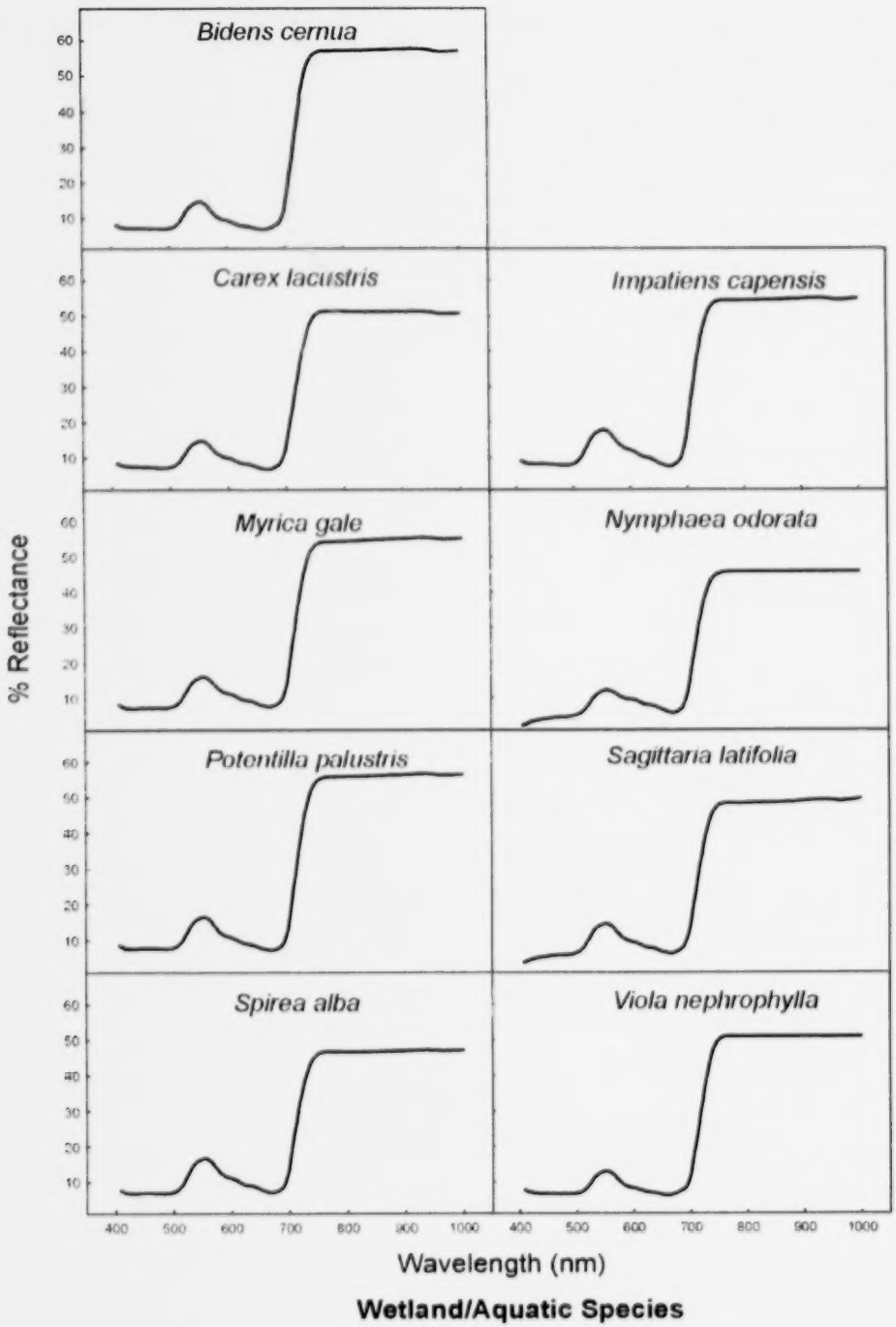

Figure 2 (con't). *Leaf spectral reflectance of Ontario species. D. Wetland/aquatic species.*

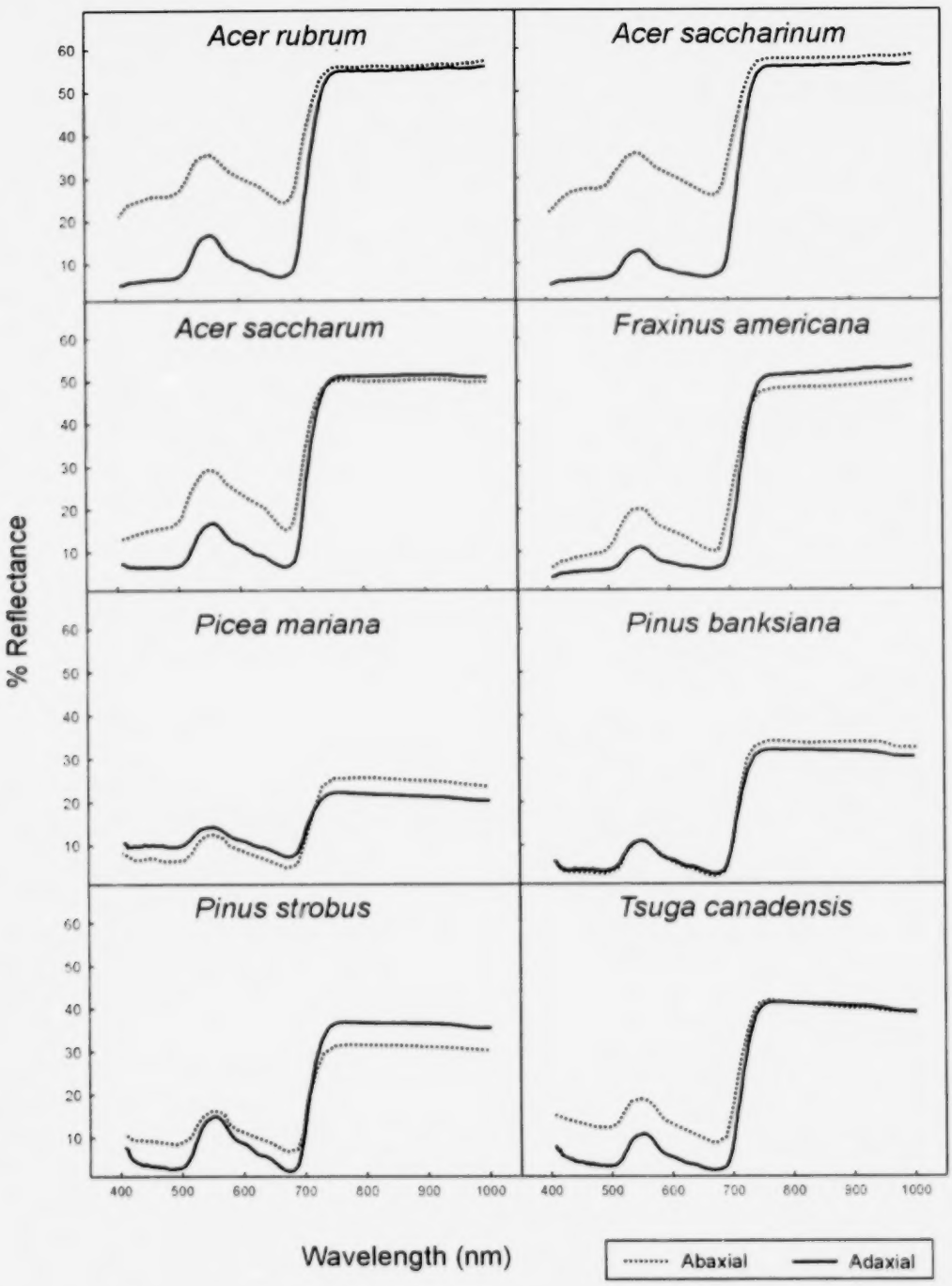

Figure 3. *Abaxial and adaxial leaf reflectance.*

Table 3. *Spectral index values for leaf spectra of 44 Ontario plant species.*

Species	FR/G	R/FR	FR/R	NDVI	FR726	FR720	PRI	RE	REIP	B/R	B/FR
Evergreen Tree/Shrub											
Abies balsamea	2.335	0.272	2.597	0.686	0.326	0.593	0.033	20.30	703.5	1.218	0.300
Picea abies	2.492	0.247	2.561	0.778	0.300	0.539	0.028	10.80	701.5	0.883	0.176
Picea glauca	1.889	0.348	2.096	0.630	0.296	0.531	0.021	11.50	700.8	0.965	0.300
Picea mariana	1.549	0.458	1.740	0.488	0.261	0.464	0.019	12.70	699.5	1.124	0.470
Pinus banksiana	2.908	0.199	3.047	0.798	0.286	0.509	0.025	10.50	705.4	0.914	0.150
Pinus resinosa	2.274	0.254	2.408	0.797	0.264	0.457	0.006	15.60	701.5	0.666	0.130
Pinus rigida	1.407	0.518	1.461	0.588	0.184	0.305	0.008	23.10	694.2	0.543	0.232
Pinus strobus	2.466	0.209	2.612	0.871	0.255	0.442	-0.008	14.10	700.8	0.737	0.110
Thuja occidentalis	2.603	0.210	2.840	0.832	0.294	0.521	-0.023	15.50	702.8	0.720	0.118
Tsuga canadensis	3.864	0.129	4.296	0.870	0.333	0.612	0.034	9.70	709.4	1.141	0.119
Deciduous Tree/Shrub											
Acer pensylvanicum	3.977	0.186	3.756	0.746	0.449	0.861	0.025	13.40	712.7	0.687	0.124
Acer rubrum	3.259	0.201	3.190	0.761	0.396	0.731	0.001	17.00	705.4	0.628	0.113
Acer saccharinum	4.247	0.167	4.080	0.768	0.476	0.916	0.010	13.30	713.4	0.705	0.116
Acer saccharum	3.026	0.222	2.768	0.763	0.334	0.594	-0.038	18.30	703.5	0.694	0.130
Alnus crispa	4.050	0.174	3.860	0.774	0.383	0.716	-0.006	13.90	712.7	0.617	0.101
Betula alleghaniensis	3.972	0.181	3.740	0.767	0.423	0.802	-0.020	13.00	714.0	0.770	0.132
Betula papyrifera	3.924	0.172	3.767	0.776	0.363	0.672	-0.007	13.90	710.7	0.721	0.115
Fagus grandifolia	4.245	0.178	3.792	0.766	0.433	0.821	0.024	13.60	712.1	0.727	0.123
Fraxinus americana	4.538	0.155	4.307	0.783	0.460	0.883	-0.002	11.50	714.7	0.717	0.110
Juglans nigra	3.589	0.195	3.485	0.745	0.378	0.704	-0.004	14.80	711.4	0.773	0.141
Larix laricina	2.295	0.242	2.519	0.806	0.265	0.459	-0.008	16.40	700.8	0.826	0.160
Populus deltoides	3.740	0.202	3.636	0.716	0.407	0.782	0.016	13.10	714.0	0.746	0.149
Populus tremuloides	4.549	0.165	4.363	0.761	0.438	0.852	0.015	11.50	718.0	0.818	0.134
Quercus rubra	3.918	0.164	3.849	0.790	0.390	0.726	-0.009	12.60	710.1	0.613	0.093
Rubus idaeus	3.432	0.214	3.135	0.745	0.362	0.667	-0.014	17.20	708.8	0.550	0.107
Salix humilis	3.553	0.187	3.538	0.772	0.360	0.670	-0.016	13.70	710.7	0.587	0.099
Tilia americana	3.914	0.194	3.687	0.729	0.431	0.825	0.008	11.80	708.0	0.724	0.138
Herbaceous/Moss/Lichen											
Aster macrophyllus	4.078	0.178	3.878	0.764	0.426	0.811	-0.005	13.00	714.7	0.642	0.110
Calamagrostis canadensis	1.906	0.365	2.045	0.595	0.333	0.602	-0.003	24.10	701.5	0.825	0.270
Cladina rangiferina	1.860	0.501	1.600	0.456	0.203	0.327	-0.003	30.60	700.2	0.711	0.325
Epilobium angustifolium	3.731	0.193	3.488	0.753	0.347	0.642	-0.009	13.80	710.7	0.620	0.111
Hypogymnia physodes	2.028	0.404	1.976	0.521	0.176	0.288	-0.003	31.20	705.4	0.953	0.351
Polytrichum juniperinum	2.819	0.294	2.478	0.664	0.221	0.374	-0.011	14.20	706.1	0.520	0.135
Pteridium aquilinum	3.174	0.213	3.103	0.755	0.283	0.505	-0.009	16.10	708.1	0.584	0.109
Trifolium repens	2.689	0.266	2.814	0.655	0.361	0.672	-0.004	17.80	709.4	0.805	0.204
Wetland/Aquatic											
Bidens cernua	3.837	0.177	3.832	0.761	0.366	0.686	0.002	14.60	713.4	0.802	0.135
Carex lacustris	3.443	0.199	3.536	0.749	0.432	0.830	-0.012	14.40	714.0	0.845	0.159
Impatiens capensis	3.134	0.231	2.930	0.733	0.312	0.560	-0.008	18.80	705.5	0.778	0.158
Myrica gale	3.341	0.214	3.145	0.736	0.326	0.593	-0.018	17.10	708.8	0.722	0.140
Nymphaea odorata	4.184	0.210	3.264	0.761	0.314	0.565	-0.060	14.50	708.1	0.501	0.092
Potentilla palustris	3.315	0.193	3.428	0.755	0.341	0.625	0.002	16.00	710.1	0.841	0.149
Sagittaria latifolia	3.303	0.209	3.324	0.741	0.361	0.672	0.002	14.50	710.7	0.596	0.115
Spirea alba	2.792	0.235	2.861	0.719	0.312	0.557	-0.006	16.30	706.1	0.733	0.154
Viola nephrophylla	3.911	0.182	3.905	0.749	0.414	0.798	0.019	12.90	715.4	0.801	0.141

Table 4. *Spectral index values for abaxial and adaxial leaf surfaces. Means of n=5 plants.*

Species	Surface	FR/G	R/FR	FR/R	NDVI	FR726	FR720	PRI	RE	REIP	B/R	B/FR
Acer rubrum	abaxial	1.568	0.524	1.624	0.397	0.393	0.721	0.007	34.23	700.8	0.905	0.457
	adaxial	3.259	0.201	3.190	0.761	0.396	0.731	0.001	17.01	705.4	0.628	0.113
Acer saccharinum	abaxial	1.604	0.522	1.660	0.381	0.461	0.874	0.008	34.17	701.5	0.931	0.480
	adaxial	4.247	0.167	4.080	0.768	0.476	0.916	0.010	13.33	713.4	0.705	0.116
Acer saccharum	abaxial	1.720	0.446	1.745	0.531	0.324	0.573	-0.006	28.74	698.8	0.741	0.298
	adaxial	3.026	0.222	2.768	0.763	0.334	0.594	-0.038	18.35	703.5	0.694	0.130
Fraxinus americana	abaxial	2.377	0.321	2.330	0.642	0.441	0.834	0.007	20.34	702.2	0.601	0.179
	adaxial	4.538	0.155	4.307	0.783	0.460	0.883	-0.002	11.52	714.7	0.717	0.110
Picea mariana	abaxial	2.092	0.301	2.295	0.680	0.289	0.517	0.022	11.23	700.8	1.047	0.274
	adaxial	1.549	0.458	1.740	0.488	0.261	0.464	0.019	12.66	699.5	1.124	0.470
Pinus banksiana	abaxial	3.074	0.182	3.119	0.837	0.285	0.507	0.033	10.88	704.8	0.839	0.119
	adaxial	2.908	0.199	3.047	0.798	0.286	0.509	0.025	10.46	705.4	0.914	0.150
Pinus strobus	abaxial	1.917	0.353	2.057	0.620	0.259	0.453	0.011	14.97	700.8	1.051	0.331
	adaxial	2.466	0.209	2.612	0.871	0.255	0.442	-0.008	14.07	700.8	0.737	0.110
Tsuga canadensis	abaxial	2.286	0.295	2.532	0.644	0.319	0.581	0.049	16.83	706.8	1.200	0.329
	adaxial	3.864	0.129	4.296	0.870	0.333	0.612	0.034	9.74	709.4	1.141	0.119

Sun versus shade effects

Shade leaves of *Acer saccharum* had slightly lower reflectance in the VIS and NIR than sun foliage (Figure 4). Several indices distinguished these leaf types, notably FR/G, FR/R, FR720, PRI, and REIP, all of which had higher values in shaded versus sun leaves (Table 3).

Leaf age effects

In *A. saccharum*, mature and intermediate-aged leaves had similar reflectance, while immature leaves produced lower green and NIR and higher orange-red reflectance (Figure 5). Young maple foliage was reddish in appearance when examined with the unaided eye, which was consistent with this observation. Index values were similar for mature and intermediate leaves, but were affected (by as much as 4-fold in the case of PRI) in immature leaves (Table 6). The only exception was the B/FR which was fairly similar in the 3 age groups.

In *Pinus strobus*, 1-year-old needles had slightly higher reflectance throughout the VIS (more notable in the green region) and NIR (Figure 5). Index values were slightly affected but PRI was 4 times higher in current-year foliage than in 1-year-old needles (Table 6).

The mature needles of *Tsuga canadensis* had the lowest reflectance in the VIS and the highest in the NIR region, while immature needles had the highest in the VIS and lowest in the NIR (Figure 5). New needles of hemlock were also lighter green in colour and were softer and thinner than mature or intermediate leaves. Differences in index values were pronounced for most of the indices, particularly PRI, which was 15 times greater in mature than in immature needles (Table 6).

In *A. saccharum* and *T. canadensis*, most indices behaved in a similar manner in response to foliar stage of maturation. The exception was FR/G, which decreased with leaf maturation in *A.*

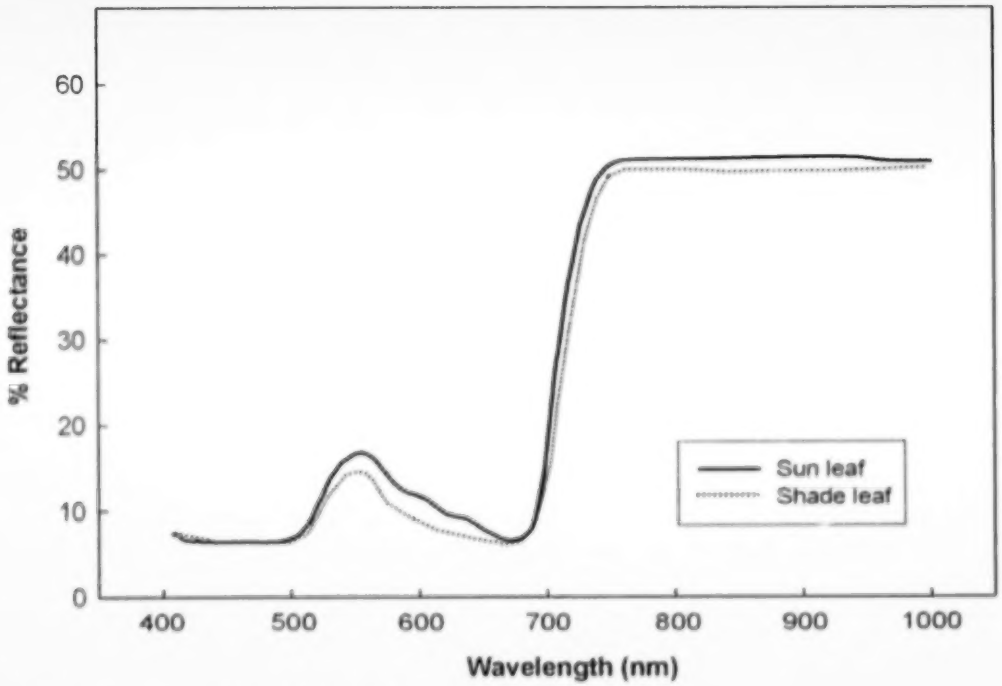

Figure 4. *Sun and shade leaf reflectance.*

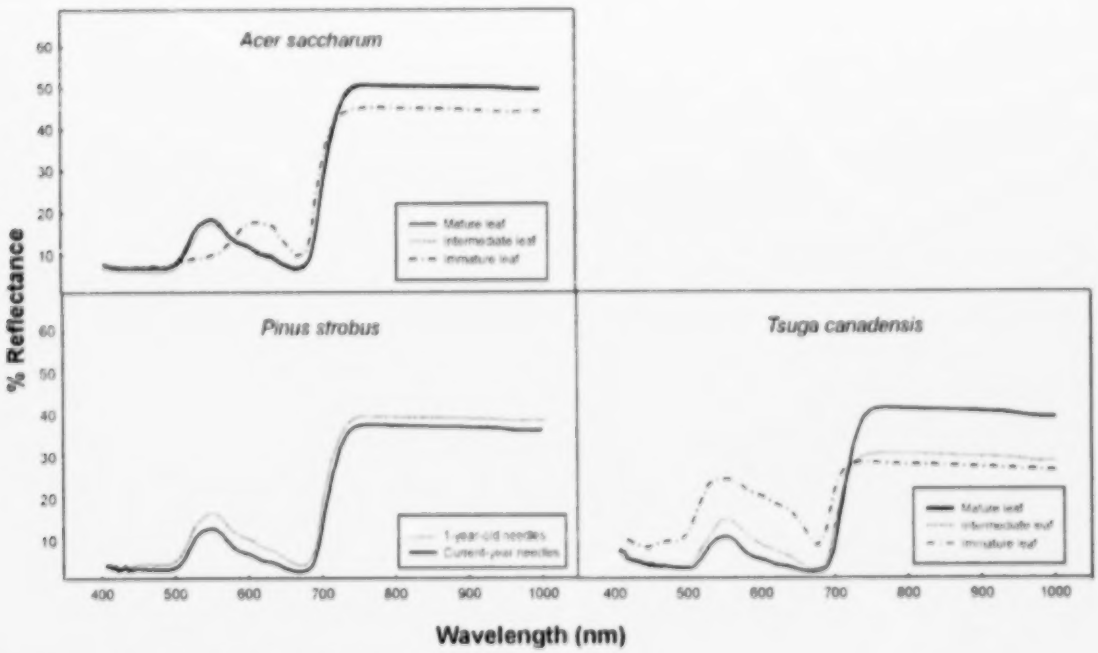

Figure 5. *Leaf age effects on reflectance.*

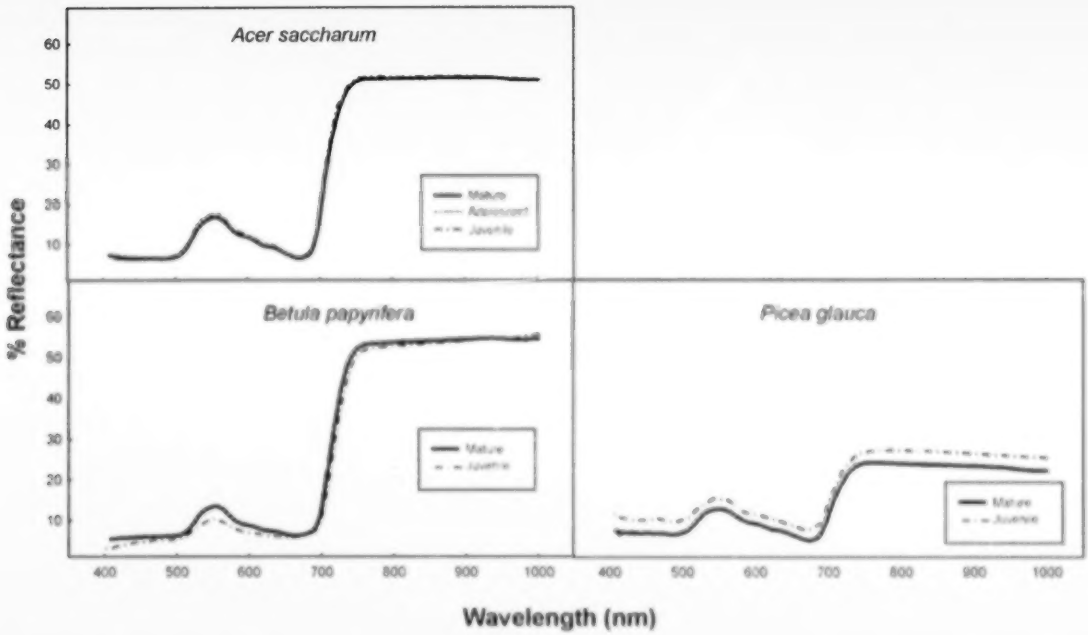

Figure 6. Tree age effects on reflectance.

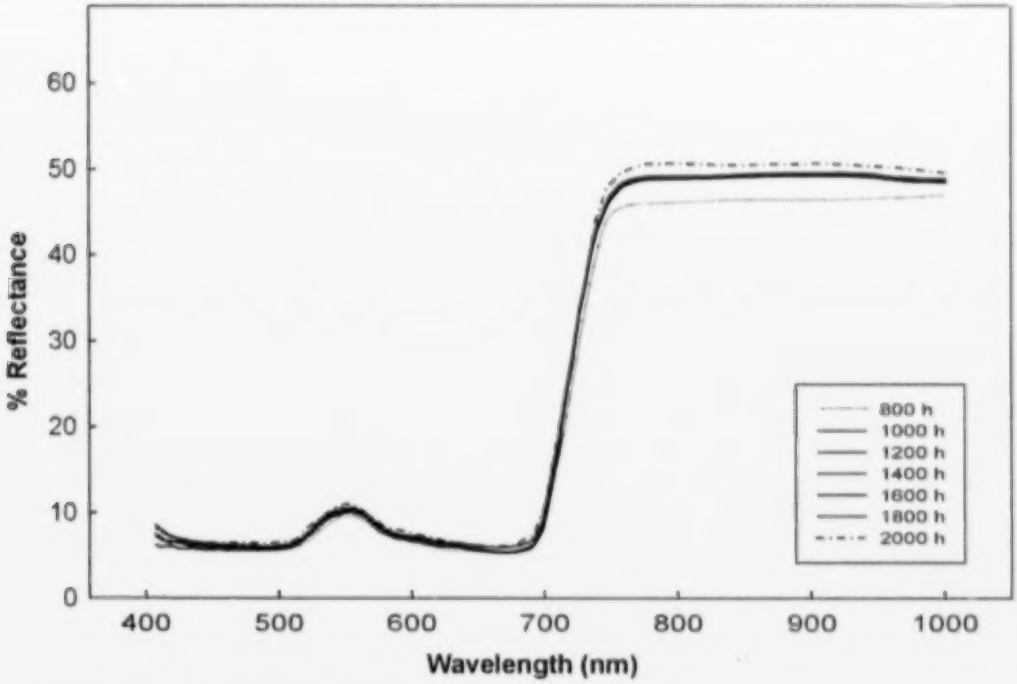

Figure 7. *Diurnal effects on reflectance.*

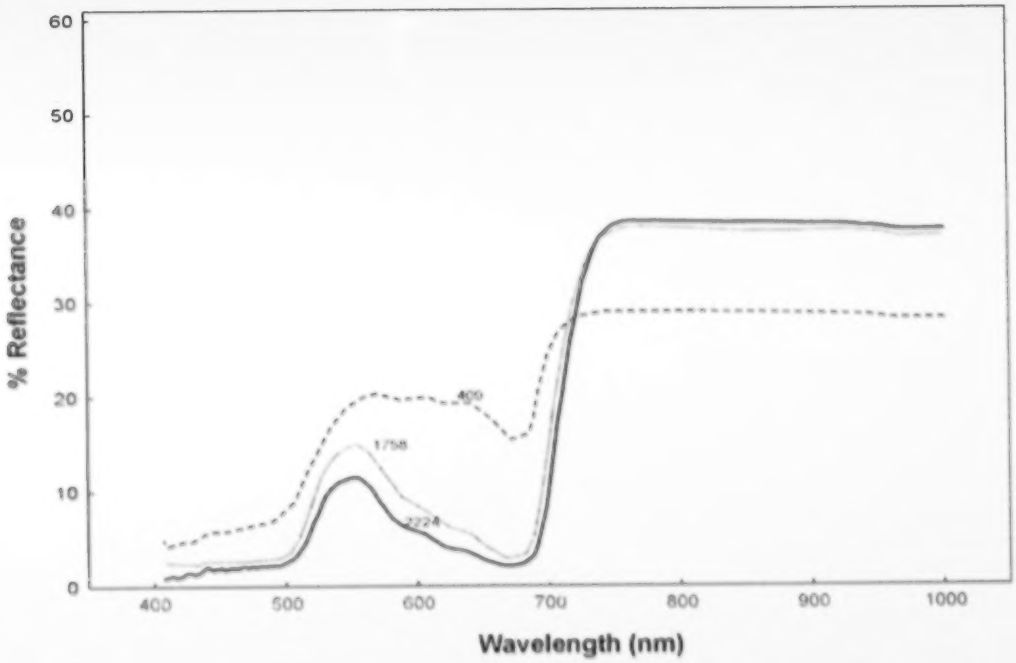

Figure 8. *Senescence effects on reflectance of* Pinus strobus. *Chlorophyll a concentrations in µg.g^{-1} DW are shown.*

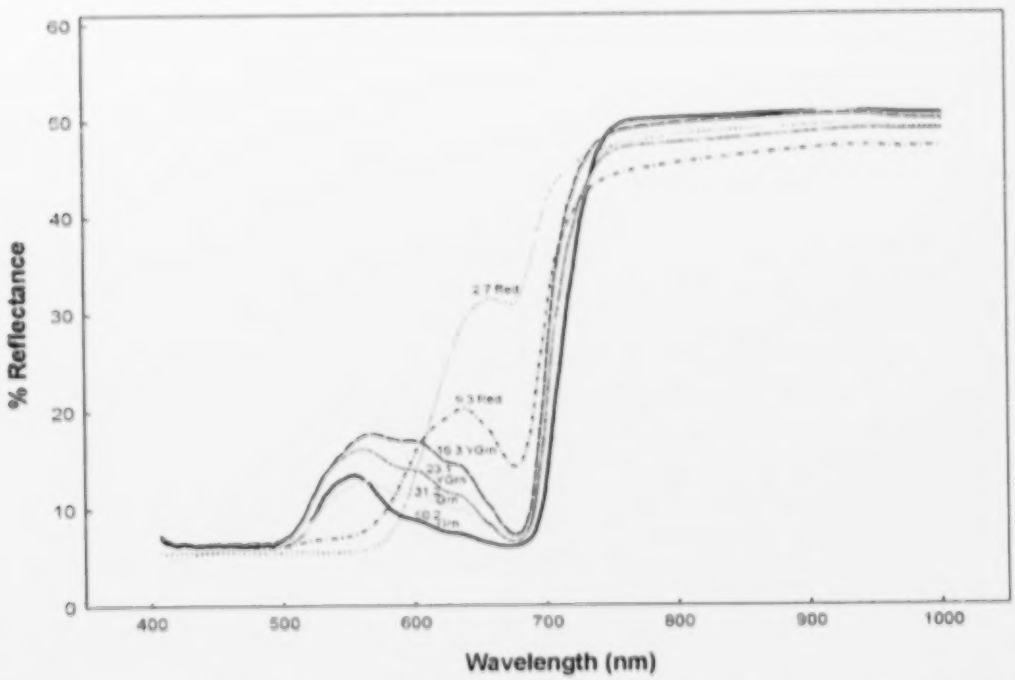

Figure 9. *Senescence effects on reflectance of* Acer saccharum. *Chlorophyll a concentrations in µg.cm^{-2} and leaf colour are shown.*

saccharum but increased in *T. canadensis*, the latter feature resulting from the much higher green and lower NIR reflectance in immature needles of hemlock.

Tree age effects

Tree age had no effect on leaf spectra of *A. saccharum*, very slight effect in *Betula papyrifera*, and a small effect in *Picea glauca* (Figure 6). Accordingly, spectral index values were quite similar among the age groups (Table 7).

Diurnal effects

Chlorophyll content in this study remained constant throughout the day, ranging from 44.2 to 45.8 on a relative scale. PPFD peaked at a value of 1826 $\mu mol.m^{-2}.s^{-1}$ observed at 1400 h, and air temperature ranged from 15°C at 800 h to 33°C at 1600 h. Reflectance increased slightly after 800 h, but more so in the NIR than in the VIS spectrum (Figure 7). A statistically significant increase was observed in B/R during the midday hours, but other indices were similar throughout the day (Table 8). Increase in B/R was the result of both higher blue and lower red reflectance. PRI tended to decrease slightly during the day, but not significantly.

Senescence stress effects

In *Pinus strobus*, reduction in chlorophyll content was manifested as an increase in the VIS and a decrease in NIR reflectance (Figure 8). Chlorosis detectable with the unaided eye (409 $\mu g.g^{-1}$ DW) produced a marked effect on leaf spectra, while previsual chlorosis (1758 $\mu g.g^{-1}$ DW) had a less pronounced but spectrally discernible effect. Correlations were very strong (R>0.90) between chlorophyll content and certain spectral indices, namely, FR/G, FR/R, REIP, and B/FR (Table 9). These indices were also well correlated with carotenoid concentrations.

In *A. saccharum*, previsual reduction in chlorophyll content was accompanied by an increase in reflectance in the yellow-orange wavebands (Figure 9). With further decline in chlorophyll, green reflectance increased then decreased, and red reflectance increased, the latter markedly so by the time leaves were bright red. NIR reflectance declined with decreasing chlorophyll, but not in a consistent manner. These assessments spanned a range in chlorophyll from 50.2 down to 2.7 $\mu g.cm^{-2}$, encompassing a colour transition from deep green to red in the autumn. The indices FR/R, REIP, and B/R were strongly correlated (R≥0.90) with chlorophyll (Table 10). The best correlation with carotenoid concentration was obtained with FR/R (R=0.82).

Potted *A. saccharum* transferred from a shade environment to full sun exhibited early senescence effects as a modest increase in reflectance from about 540 to 650 nm and a small blue shift in REIP (Figure 10). Index values were significantly lower in the foliage of transferred plants for FR/R, FR726, FR720, PRI, REIP, and B/R, and higher for RE (Table 11). The chlorophyll fluorescence feature F_v/F_{max} was lower in transferred plants, as were chlorophyll (previsual) and carotenoid concentrations. The photosynthesis features P_n, COND, and CINT were similar for shaded and transferred plants.

Herbicide and girdling stress effects

Herbicide and girdling produced an increase in VIS reflectance, but effects were not consistent across species for the NIR region (Figure 11). *Picea mariana* was more sensitive to girdling than *Pinus banksiana* or 1-year-old needles of *Pinus resinosa*, as evidenced in a higher green and NIR reflectance. Current-year needles of *P. resinosa* were more sensitive to herbicide than older needles, especially to imazapyr, where a pronounced increase in VIS reflectance was observed.

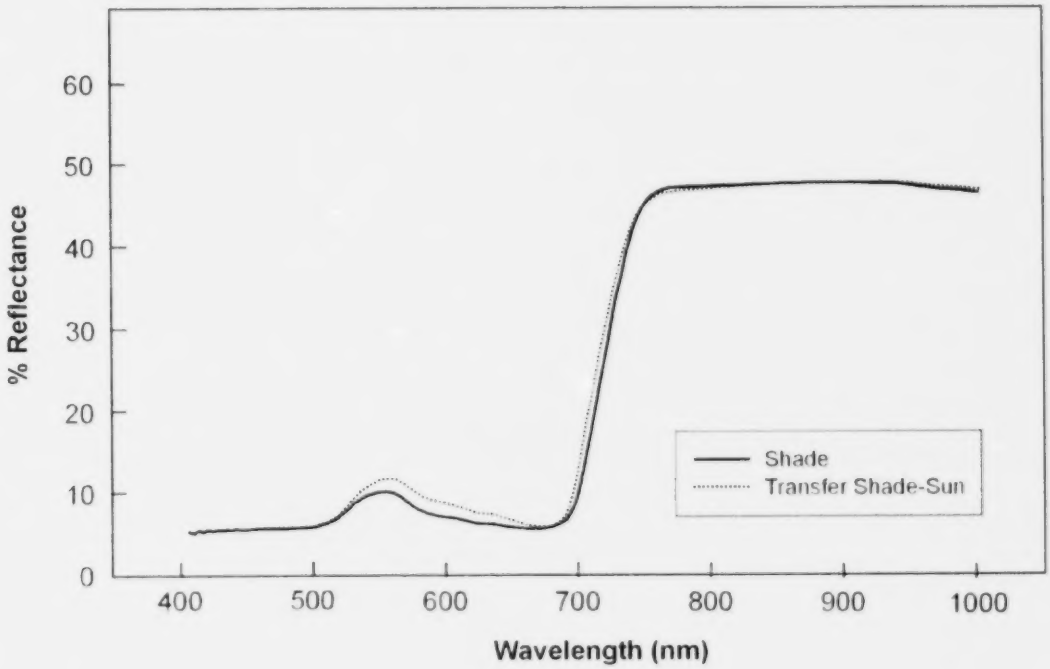

Figure 10. *Reflectance of shade-grown or transferred (shade to sun) seedlings of* Acer saccharum.

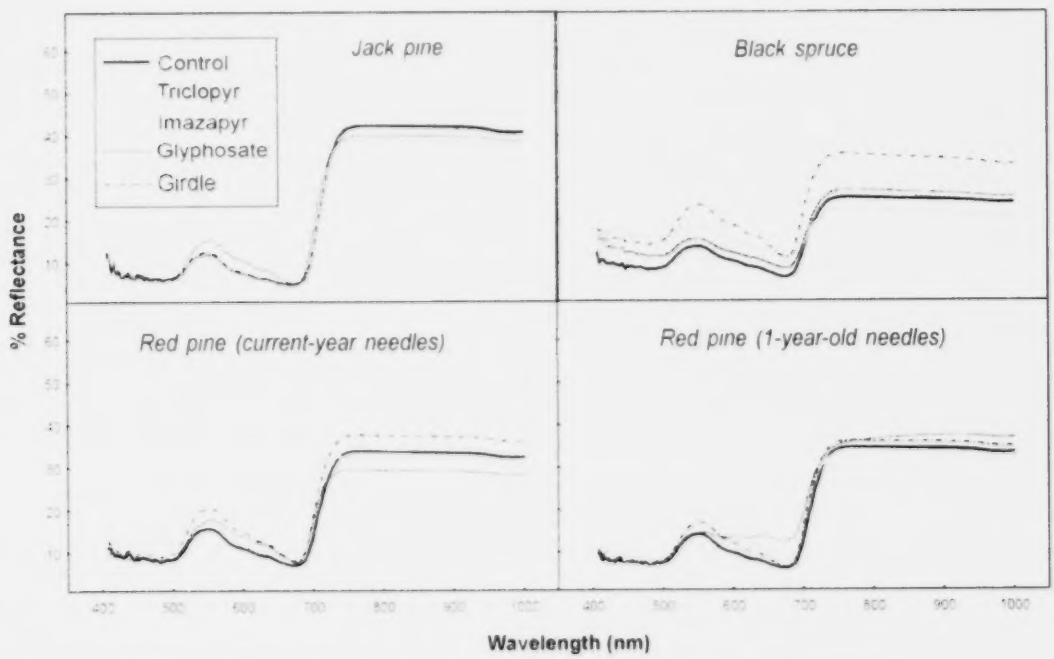

Figure 11. *Reflectance of conifer species subjected to herbicide and girdling treatments.*

Table 5. *Spectral index values for sun and shade leaves of* Acer saccharum. *Mature trees and adaxial leaf surfaces were used. Means of n=5 plants.*

Leaf type	FR/G	R/FR	FR/R	NDVI	FR726	FR720	PRI	RE	REIP	B/R	B/FR
Sun	3.026	0.222	2.768	0.763	0.334	0.594	-0.038	18.35	703.5	0.694	0.130
std error	0.059	0.007	0.101	0.002	0.012	0.026	0.007	0.70	0.7	0.023	0.002
Shade	3.493	0.206	3.222	0.746	0.398	0.740	0.030	15.50	706.1	0.695	0.131
std error	0.234	0.011	0.173	0.006	0.016	0.036	0.003	0.90	1.3	0.025	0.003

Table 6. *Spectral index values for leaves of different age, size, or type. Means of n=5 plants.*

Leaf type	FR/G	R/FR	FR/R	NDVI	FR726	FR720	PRI	RE	REIP	B/R	B/FR
Acer saccharum											
Mature	2.784	0.254	2.501	0.743	0.307	0.540	-0.025	20.42	702.1	0.664	0.140
Intermediate	2.896	0.251	2.449	0.759	0.302	0.528	-0.034	20.66	701.5	0.629	0.128
Immature	4.922	0.436	1.633	0.641	0.251	0.422	-0.106	27.77	696.2	0.447	0.157
Pinus strobus											
One-year-old	2.376	0.257	2.379	0.792	0.265	0.462	-0.010	16.58	700.8	0.555	0.110
Current-year	2.902	0.186	3.076	0.845	0.292	0.521	0.031	12.17	703.5	0.678	0.097
Tsuga canadensis											
Mature	3.864	0.129	4.296	0.870	0.333	0.612	0.034	9.74	709.4	1.141	0.119
Intermediate	2.081	0.274	2.257	0.804	0.228	0.386	-0.017	13.61	700.2	0.737	0.146
Immature	1.153	0.645	1.254	0.484	0.103	0.165	-0.002	22.60	691.7	0.545	0.299

Table 7. *Spectral index values for trees of different age. Means of n=5 plants.*

Leaf type	FR/G	R/FR	FR/R	NDVI	FR726	FR720	PRI	RE	REIP	B/R	B/FR
Acer saccharum											
Mature	3.026	0.222	2.768	0.763	0.334	0.594	-0.038	18.35	703.5	0.694	0.130
Adolescent	2.967	0.221	2.767	0.767	0.338	0.601	-0.034	18.41	702.8	0.709	0.131
Juvenile	2.984	0.234	2.668	0.760	0.309	0.542	-0.036	19.65	703.5	0.682	0.131
Betula papyrifera											
Mature	3.924	0.172	3.767	0.776	0.363	0.672	-0.007	13.95	710.7	0.721	0.115
Juvenile	4.900	0.151	4.622	0.785	0.422	0.813	0.000	11.02	717.3	0.639	0.096
Picea glauca											
Mature	1.889	0.348	2.096	0.630	0.296	0.531	0.021	11.50	700.8	0.965	0.300
Juvenile	1.802	0.384	2.008	0.566	0.305	0.551	0.023	13.64	700.8	1.109	0.386

Table 8. *Spectral index values of* Acer saccharum *at different times of the day. Means of n=5 plants.*

Time of day	FR/G	R/FR	FR/R	NDVI	FR726	FR720	PRI	RE	REIP	B/R	B/FR
800	4.717	0.154	4.626	0.780	0.577	1.166	0.012	9.57	720.1	0.943 b	0.149
1000	4.671	0.144	4.738	0.799	0.547	1.088	-0.005	10.12	717.3	0.937 b	0.135
1200	4.715	0.138	4.954	0.800	0.528	1.039	0.002	9.52	716.8	1.018 a	0.143
1400	4.555	0.143	4.750	0.796	0.514	1.006	-0.004	10.00	716.7	1.051 a	0.151
1600	4.650	0.142	4.817	0.797	0.523	1.028	-0.001	9.81	716.8	1.069 a	0.153
1800	4.514	0.155	4.533	0.777	0.545	1.084	0.006	10.53	717.9	0.918 b	0.145
2000	4.506	0.158	4.498	0.774	0.547	1.094	0.008	10.99	717.9	0.911 b	0.146

Table 9. *Spectral index values of* Pinus strobus *with different concentrations of chlorophyll. Examples from 3 plants are listed. Correlation coefficient (R) for chlorophyll a vs. spectral index is shown (10 trees, 3 fascicles per tree).*

Chl *a* content µg.g^{-1} DW)	FR/G	R/FR	FR/R	NDVI	FR726	FR720	PRI	RE	REIP	B/R	B/FR
2224	3.338	0.148	3.479	0.885	0.300	0.536	0.034	10.97	704.1	0.487	0.055
1758	2.559	0.219	2.580	0.847	0.258	0.448	0.010	14.63	700.8	0.446	0.072
409	1.543	0.700	1.251	0.385	0.280	0.484	-0.114	24.30	677.6	0.314	0.188
R chlorophyll a	0.93	-0.90	0.94	0.88	0.01	0.18	0.86	-0.78	0.93	0.24	-0.94
R chlorophyll b	0.90	-0.86	0.91	0.84	0.02	0.19	0.83	-0.76	0.92	0.19	-0.93
R carotenoids	0.93	-0.89	0.93	0.86	0.00	0.17	0.83	-0.77	0.94	0.28	-0.94

Table 10. *Spectral index values of* Acer saccharum *with different concentrations of chlorophyll and various stages of autumn coloration. Examples from 6 plants are listed. Correlation coefficient (R) for chlorophyll a vs. spectral index is shown (10 plants, 2 leaves per plant).*

Colour & chl *a* content (µg.cm^{-2})		FR/G	R/FR	FR/R	NDVI	FR726	FR720	PRI	RE	REIP	B/R	B/FR
Deep green	50.2	3.644	0.182	3.454	0.774	0.401	0.741	-0.011	14.17	706.9	0.820	0.135
Dull green	31.2	3.780	0.271	2.551	0.733	0.388	0.691	-0.089	19.06	701.9	0.589	0.122
Yellow-green	23.1	2.984	0.292	2.153	0.745	0.332	0.582	-0.072	21.65	697.5	0.612	0.143
Yellow-green	16.3	2.934	0.343	1.875	0.736	0.297	0.503	-0.136	26.04	697.5	0.495	0.132
Red	9.3	6.135	0.548	1.492	0.532	0.414	0.725	-0.117	30.50	694.2	0.348	0.144
Red	2.7	8.454	0.810	1.150	0.225	0.542	0.985	-0.061	41.56	689.7	0.163	0.121
R chlorophyll a		**-0.42**	**-0.85**	**0.99**	**0.73**	**-0.14**	**-0.04**	**0.62**	**-0.92**	**0.98**	**0.94**	**0.32**
R chlorophyll b		**-0.30**	**-0.80**	**0.98**	**0.67**	**-0.04**	**0.05**	**0.68**	**-0.88**	**0.96**	**0.90**	**0.37**
R carotenoids		**0.04**	**-0.47**	**0.82**	**0.30**	**0.36**	**0.44**	**0.72**	**-0.62**	**0.75**	**0.63**	**-0.54**

Table 11. *Spectral indices, pigment concentrations, chlorophyll fluorescence, and photosynthesis in* Acer saccharum *under artificially induced senescence in late summer. N=5 plants, 2 leaves per plant. Means in a row with the same letter are not significantly different (p<0.05).*

Feature	Shade	Sun
Spectral index		
FR/G	4.464 a	3.703 a
R/FR	0.167 a	0.227 a
FR/R	4.122 a	2.970 b
NDVI	0.770 a	0.762 a
FR726	0.535 a	0.412 b
FR720	1.058 a	0.761 b
PRI	0.001 a	-0.080 b
RE	13.42 b	19.26 a
REIP	716.2 a	705.9 b
B/R	0.812 a	0.703 b
B/FR	0.135 a	0.135 a
Chlorophyll fluorescence		
F_v/F_{max}	0.75 a	0.60 b
Pigment concentration		
Chl *a*, $\mu g.cm^{-2}$	70.92 a	43.24 b
Chl *b*, $\mu g.cm^{-2}$	22.50 a	14.01 b
Carotenoid, $\mu g.cm^{-2}$	17.84 a	14.50 b
Photosynthesis		
P_n, $\mu mol.m^{-2}.s^{-1}$	2.239 a	2.389 a
COND, $mol.m^{-2}.s^{-1}$	0.083 a	0.091 a
CINT, ppm	249.0 a	231.5 a

In *P. banksiana*, spectral features responded earlier than chlorophyll *a*, *b*, or carotenoids (Table 12). Indices pointed to adverse effects of glyphosate by a decrease in FR/G, FR/R, FR726, FR720, PRI, REIP, and B/R, and an increase in R/FR and RE. This was accompanied by a decrease in F_v/F_{max}. Subsequent growth and percent browning assessed in the autumn were consistent with the spectral and chlorophyll fluorescence results measured during the summer (prior to the appearance of visual effects). Strong correlations (R>0.90) were found between early spectral features and later growth (also chlorophyll fluorescence and growth) for all indices except NDVI and B/FR. Neither chlorophyll content nor carotenoid concentration measured during the summer were indicative of later effects on growth and browning (Table 12).

In *P. mariana*, in addition to chlorophyll fluorescence, the spectral indices B/R and B/FR were especially well correlated (R≥0.85) to later growth and browning, while chlorophyll and carotenoids were poorly correlated (R≤0.44) (Table 13).

Similarly, in *P. resinosa*, spectral and fluorescence features were better correlated to growth and browning than chlorophyll and carotenoids (Table 14). Correlation coefficients exceeded 0.80 for all indices except FR/G and B/FR.

Pooling all species and treatments (total of 40 group means), both PRI and F_v/F_{max} produced reasonable correlations (R≥0.61) to subsequent growth and browning (Table 15).

Table 12. *Spectral indices, pigment concentrations, chlorophyll fluorescence (CF), stem growth and percent browning of* **Pinus banksiana** *exposed to herbicide or girdling stress. Indices, pigments and CF were assessed in July 1998 (8 weeks after treatment, mean of 5 trees), growth and browning in October 1998 (mean of all surviving trees). Means in a row with the same letter(s) not significantly different p<0.05. Correlation coefficient (R) shown for indices, pigments, CF vs. growth, % brown.*

	TREATMENT						
	Triclopyr	Control	Imazapyr	Girdled	Glyphosate	*R* growth	*R* % brown
Spectral Index							
FR/G	3.215 a	3.457 a	3.437 a	3.306 a	2.602 b	**0.97**	**-0.99**
R/FR	0.202 b	0.189 b	0.186 b	0.203 b	0.273 a	**-0.96**	**0.99**
FR/R	3.298 a	3.381 a	3.408 a	3.139 a	2.412 b	**0.93**	**-0.98**
NDVI	0.755 a	0.778 a	0.785 a	0.769 a	0.765 a	**0.59**	**-0.71**
FR726	0.329 a	0.324 a	0.325 a	0.308 a	0.255 b	**0.94**	**-0.98**
FR720	0.602 a	0.588 a	0.592 a	0.555 a	0.439 b	**0.94**	**-0.98**
PRI	0.038 a	0.043 a	0.042 a	0.045 a	-0.019 b	**1.00**	**-0.98**
RE	11.80 b	12.33 b	12.05 b	13.39 b	17.13 a	**-0.94**	**0.98**
REIP	709.4 a	708.1 a	708.7 a	706.8 a	702.1 b	**0.92**	**-0.97**
B/R	1.054 a	1.003 a	1.034 a	1.001 a	0.811 b	**0.97**	**-0.97**
B/FR	0.190 a	0.166 a	0.169 a	0.175 a	0.172 a	**-0.19**	**0.39**
Chlorophyll fluorescence							
F_v/F_{max}	0.82 a	0.84 a	0.82 a	0.83 a	0.53 b	**1.00**	**-0.99**
Pigment concentration							
Chl *a* µg g^{-1} DW	2300 a	2159 a	2330 a	1867 a	1826 a	**0.51**	**-0.64**
Chl *b* µg g^{-1} DW	657.0 a	632.9 a	674.3 a	544.5 a	526.6 a	**0.55**	**-0.68**
Carotenoid µg g^{-1} DW	519.7 a	492.2 a	530.2 a	431.7 a	431.4 a	**0.45**	**-0.58**
Final morphology							
Stem growth cm	41.2 a	40.6 a	33.0 a	45.3 a	-43.3 b		
Brown (%)	4.4 b	0.0 b	5.4 b	6.7 b	39.4 a		

Table 13. *Spectral indices, pigment concentrations, chlorophyll fluorescence (CF), stem growth and percent browning of Picea mariana exposed to herbicide or girdling stress. Indices, pigments and CF were assessed in July 1998 (8 weeks after treatment, mean of 5 trees), growth and browning in October 1998 (mean of all surviving trees). Means in a row with the same letter(s) not significantly different p<0.05. Correlation coefficient (R) shown for indices, pigments, CF vs. growth, % brown.*

	TREATMENT						
	Triclopyr	Control	Imazapyr	Girdled	Glyphosate	*R* growth	*R* % brown
Spectral Index							
FR/G	1.412 b	1.851 a	1.456 b	1.511 ab	1.725 ab	**0.52**	**-0.29**
R/FR	0.522 a	0.380 b	0.506 a	0.475 ab	0.444 ab	**-0.72**	**0.53**
FR/R	1.634 b	1.991 a	1.626 b	1.639 b	1.864 ab	**0.50**	**-0.28**
NDVI	0.382 b	0.571 a	0.427 b	0.509 ab	0.486 ab	**0.85**	**-0.73**
FR726	0.273 ab	0.291 a	0.252 ab	0.242 b	0.285 ab	**0.27**	**-0.03**
FR720	0.489 ab	0.521 a	0.444 ab	0.419 b	0.509 ab	**0.25**	**-0.02**
PRI	0.031 a	0.022 a	0.019 a	-0.0004 b	0.021 a	**-0.04**	**0.21**
RE	16.10 ab	12.61 b	13.74 b	21.55 a	14.36 b	**-0.05**	**-0.09**
REIP	701.5 a	701.5 a	699.5 ab	697.5 b	701.5 a	**0.08**	**0.15**
B/R	1.340 a	1.042 c	1.289 ab	1.110 bc	1.309 a	**-0.96**	**0.96**
B/FR	0.662 a	0.365 b	0.607 a	0.466 ab	0.548 ab	**-0.93**	**0.85**
Chlorophyll fluorescence							
F_v/F_{max}	0.83 a	0.83 a	0.79 a	0.78 a	0.75 a	**0.89**	**-0.90**
Pigment concentration				746 c	1832 b	**0.44**	**-0.23**
Chl *a* µg g^{-1} DW	1706 b	2416 a	1123 c				
Chl *b* µg g^{-1} DW	459.8 ab	664.5 a	314.7 bc	196.42 c	526.2 ab	**0.39**	**-0.17**
Carotenoid µg g^{-1} DW	400.0 bc	545.1 a	295.8 cd	212.2 d	456.3 ab	**0.37**	**-0.15**
Final morphology							
Stem growth cm	29.6 a	34.7 a	-43.8 b	-12.4 ab	-58 b		
Brown (%)	0.0 c	0.0 c	41.0 ab	20.0 bc	61.9 a		

Table 14. *Spectral indices, pigment concentrations, chlorophyll fluorescence (CF), stem growth and percent browning of Pinus resinosa exposed to herbicide or girdling stress. Indices, pigments and CF were assessed in July 1998 (8 weeks after treatment, mean of 5 trees), growth and browning in October 1998 (mean of all surviving trees). Means in a row with the same letter(s) not significantly different p<0.05. Correlation coefficient (R) shown for indices, pigments, CF vs. growth, % brown.*

	TREATMENT						
	Triclopyr	Control	Imazapyr	Girdled	Glyphosate	*R* growth	*R* % brown
Spectral Index							
FR/G	2.345 a	2.435 a	2.085 a	2.103 a	2.558 a	**0.60**	**-0.60**
R/FR	0.289 a	0.272 a	0.443 a	0.314 a	0.441 a	**-0.95**	**0.97**
FR/R	2.432 ab	2.531 a	1.806 b	2.208 ab	1.955 b	**0.89**	**-0.91**
NDVI	0.670 a	0.691 a	0.576 a	0.689 a	0.543 a	**0.94**	**-0.98**
FR726	0.302 a	0.301 a	0.407 a	0.266 a	0.457 a	**-0.93**	**0.96**
FR720	0.538 a	0.537 a	0.715 a	0.462 a	0.827 a	**-0.91**	**0.94**
PRI	0.025 a	0.029 a	-0.027 a	-0.003 a	-0.05 a	**0.84**	**-0.84**
RE	14.64 a	13.55 a	18.13 a	16.21 a	20.37 a	**-0.81**	**0.84**
REIP	704.8 a	704.9 a	696.8 a	700.8 a	700.8 a	**0.88**	**-0.87**
B/R	1.079 a	1.024 a	0.777 ab	0.898 ab	0.668 b	**0.87**	**-0.83**
B/FR	0.278 a	0.245 ab	0.274 a	0.235 ab	0.219 b	**-0.35**	**0.50**
Chlorophyll fluorescence							
F_v/F_{max}	0.83 a	0.84 a	0.49 b	0.78 a	0.33 b	**0.97**	**-0.99**
Pigment concentration							
Chl *a* µg.g^{-1} DW	1820 ab	2773 a	818.8 b	1336 b	868.2 b	**0.63**	**-0.70**
Chl *b* µg.g^{-1} DW	503.9 a	806.2 a	272.0 a	367.3 a	279.8 a	**0.59**	**-0.65**
Carotenoid µg.g^{-1} DW	422.0 ab	545.5 a	248.1 b	325.9 b	234.5 b	**0.67**	**-0.71**
Final morphology							
Stem growth cm	55 a	21.5 a	-117.3 b	28.8 a	-129.3 b		
Brown (%)	0.0 b	0.0 b	85.0 a	0.0 b	100 a		

	TREATMENT	
	R growth	*R* % brown
Spectral Index		
FR/G	0.32	-0.26
R/FR	-0.49	0.44
FR/R	0.46	-0.39
NDVI	0.38	-0.34
FR726	0.00	-0.01
FR720	0.03	-0.04
PRI	0.64	-0.61
RE	-0.27	0.23
REIP	0.54	-0.50
B/R	0.09	-0.07
B/FR	-0.27	0.25
Chlorophyll fluorescence		
F_v/F_{max}	0.69	-0.69
Pigment concentration		
Chl *a* µg.g^{-1} DW	0.50	-0.45
Chl *b* µg.g^{-1} DW	0.45	-0.41
Carotenoid µg.g^{-1} DW	0.42	-0.36

Table 15. *Correlation between spectral indices, pigment concentrations, and chlorophyll fluorescence (CF), vs. stem growth and percent browning in conifers exposed to herbicide or girdling stress. Indices, pigments, and CF were assessed in July 1998 (8 weeks after treatment), growth and browning in October 1998 (mean of all surviving trees). Correlation coefficient combines 8 stocklots* (Pinus resinosa, P. banksiana, Picea glauca, P. mariana) *and 5 treatments (control, triclopyr, imazapyr, glyphosate, girdled) with 5 trees sampled per combination.*

Discussion

Factors affecting nonvisual leaf reflectance

Leaf reflectance was influenced by most of the factors studied here, including species, leaf side, leaf age, and stress; and to a lesser degree light acclimation, tree age, and time of day.

Factors known to affect nonvisual leaf reflectance include chemical constituents such as pigments, lignin, cellulose, proteins, nitrogen, and water content; structural factors such as leaf thickness, palisade and spongy mesophyll thickness, and cuticle structure; seasonal and diurnal factors; developmental factors such as leaf age; and species (Ourcival et al. 1999, Knapp and Carter 1998, Middleton et al. 1998, Roberts et al. 1998, Zwiggelaar 1998, Dendron Resource Surveys 1997, Peñuelas et al. 1995a, Yoder and Pettigrew-Crosby 1995, Carter 1994, Carter 1993, Miller et al. 1991, Clark and Lister 1975).

Chemical constituents

The important pigments that affect absorption have been reviewed by others (Zwiggelaar 1998, Stockburger and Mitchell 1999) and include:

- chlorophyll *a*: 435, 670-680, 740 nm;
- chlorophyll *b*: 480, 650 nm;
- α-carotenoid: 420, 440, 470 nm;
- ß-carotenoid: 425, 450, 480 nm;
- anthocyanin: 400-550 nm;
- lutein: 425, 445, 475 nm;
- violaxanthin: 425, 450, 475 nm.

Most leaves contain a combination of these pigments (and absorption peaks are fairly broad), hence, absorption spectra do not have sharply defined peaks.

Our results support previous findings of strong correlations between narrow-band features near the red edge and chlorophyll content (Gitelson and Merzlyak 1996, Belanger et al. 1995, Vogelmann et al. 1993, Horler et al. 1983). But in addition to the indices REIP and FR/R (i.e., R750/R700), those involving green and blue portions of the spectrum also showed promise. In *Pinus strobus*, FR/G (i.e., R750/R550) and B/FR (i.e., R440/R740) were highly correlated with chlorophyll, as was B/R (i.e., R440/R690) in *Acer saccharum*.

Gitelson and Merzlyak (1996) found maximum sensitivity to chlorophyll *a* concentration occurred at 550 to 560 nm and 700 to 710 nm in horse chestnut (*Aesculus hippocastanum* L.) and Norway maple (*Acer platanoides* L.) trees. They reported that the spectral bands ranging from 400 to 480 nm and above 730 nm were not sensitive and suggested these wavelengths could be used as references in the vegetation indices. Indices such as R750/R550 and R750/R700 were reported to be directly proportional (correlation $R^2>0.95$) to chlorophyll concentration. Another narrow waveband shown to correlate well with changes in leaf chlorophyll content is the green peak region, e.g., 604 to 606 nm (Carter 1994).

Here, some correlation was found between spectral indices and carotenoid concentrations in *Pinus strobus* and *A. saccharum*, and the relationship was stronger in pine. The index FR/R was well correlated with carotenoids for both species, but given the strong relationship between this index and chlorophyll content, and that chlorophyll and carotenoids are correlated, it is possible the relationship with carotenoids was either indirect or coincidental.

Peñuelas et al. (1997a, 1995b) reported that PRI was related to carotenoid-chlorophyll *a* ratio and with xanthophyll-chl *a* ratio. They also found that

carotenoids/chlorophyll *a* ratio could be estimated by the index 4.44-6.77 $\exp^{[-0.48\,(R800-R445)/(R800-R680)]}$. This index apparently minimizes confounding effects of leaf surface and mesophyll structure. In our study, the correlation coefficient was at least 0.72 between PRI and carotenoid concentration, but R values were higher with other indices such as FR/R and REIP. The differences between our findings and those reported by others may have ensued partly from our small sample sizes. However, it is also likely that the PRI spectral regions (green bands) are influenced by anthocyanin pigments, which are known to absorb in the green (Swain 1965), whereas carotenoids tend to absorb in the blue and violet bands (Weedon 1965). Carotenoids also absorb concurrently with chlorophyll in the 300 to 500 nm spectral region (Peñuelas et al. 1995a).

A diurnally based change in the ratio of blue to red reflectance was observed here in maple. The value of this index increased during the day, a result of both an increase in blue and a decrease in red reflectance which was not accompanied by changes in chlorophyll concentration. Apparent reflectance in the blue region may be influenced by concentrations of both carotenoid pigments and blue-fluorescence-producing biochemicals such as ferulic acid (Lichtenthaler and Schweiger 1998) and NADPH (Chappelle et al. 1991). Changes in red reflectance could proceed, at least in part, from quenching of chlorophyll fluorescence at midday (Zarco-Tejada et al. *a*). Diurnal spectral patterns have also been reported for the green-based spectral index PRI, which may reflect carotenoid and possibly other pigment levels (Peñuelas et al. 1995a). A trend (though not statistical) for reduced PRI values at midday was also observed in our study.

There is evidence in the literature that variations in other leaf biochemical contents, such as cellulose, lignins, proteins and nitrogen can also be detected through leaf reflectance spectra, typically in the short wave infrared region (e.g., 1200 to 2400 nm) (Yoder and Pettigrew-Crosby 1995). Most studies have emphasized canopy-level relationships (Dawson et al. 1999, Martin and Aber 1997, Gastellu-Etchegorry et al. 1995, Matson et al. 1994, Wessman et al. 1989, Peterson et al. 1988, Wessman et al. 1988) and results have not always been positive (Smith and Curran 1995, Zagolski et al. 1996). Although leaf lignin and cellulose are unlikely to change rapidly due to stress, these features may be useful for measuring long-term stress or variation in the forest (Matson et al. 1994). Martin and Aber (1997) noted few significant differences in foliar N and lignin between July and mid-September (they sampled from the end of June to mid-October), thus samples collected during this period could be used in conjunction with remote sensing data acquired during the same period.

Leaf structure

Leaf structural and developmental features are key drivers of leaf-based spectral characteristics. Middleton et al. (1998) found significant differences for adaxial versus abaxial leaf surfaces of *Pinus banksiana, Picea mariana,* and *Populus tremuloides.* Adaxial surfaces of foliage were suggested to be likely the most important because these dominate the view from above. Species differences among tree overstories were distinctly expressed in the adaxial NIR spectra, but no species effect on NIR was evident in the understories across sites. Overstory species differed especially in the adaxial blue spectrum. Abaxial surfaces of conifer needles had higher simple ratios (R800/R670) than adaxial surfaces. This morphology may represent an adaptation to protect tissue with higher photosynthetic potential from direct irradiation over a wide range of conditions occurring through the year.

Clark and Lister (1975) studied the relationship of cuticle structure to the visible and ultraviolet spectral properties of needles from the conifers *Pseudotsuga menziesii, Picea sitchensis,* and

Picea pungens. They found that the bluish appearance and low relative efficiencies of blue light in photosynthesis of *P. pungens* resulted from selective enhanced reflection of blue and ultraviolet light caused by the presence of epicuticular wax deposits. This allows these plants to tolerate high light intensities, particularly of shorter wavelengths, without damage.

Ourcival et al. (1999) reported on the relationships between reflectance and anatomical and biochemical properties in *Quercus ilex* leaves. They tested the hypothesis that spectral information on fresh leaves contains not only information about leaf biochemical composition, but also information about leaf anatomy, which could tell us more about how plants work. They looked at the thickness of tissues, cuticle, upper epidermis, palisade mesophyll, and spongy mesophyll. Due to the low variations of epidermis plus cuticle and spongy mesophyll in all leaves, correlations between spectra and these were not significant. Conversely, palisade mesophyll and total thickness were strongly correlated with reflectance spectra. Sun-leaf versus shade-leaf ratios for the palisade and spongy mesophyll thicknesses were 3.36 and 2.31, respectively. For this and other oaks, palisade mesophyll in sun leaves was composed of 2 to 3 layers of cells, whereas in shade leaves it was composed of a single layer.

Knapp and Carter (1998) found that overall leaf thickness was the best predictor of NIR reflectance and internal light scattering, however, differences in leaf longevity and growth form (e.g., as a result of sun/shade habitat) may explain the lack of consistent pattern in leaf optics.

While leaf thickness may be an important driver of leaf-level spectral characteristics, this feature is not expected to be of critical importance in canopy assessments. Some authors have undertaken to remove the confounding influence of leaf thickness in laboratory studies by using optically thick stacked layers of leaves either by measurement technique (Datt 1999, Luther 1999, Miller et al. 1991, Boyer et al. 1988) or by simulation with infinite reflectance formulae (Zarco-Tejada et al. *b*).

Using stacked layers of leaves, Miller et al. (1991) studied the influence of seasonal patterns in leaf reflectance red edge characteristics in 10 species, including *Acer saccharum*. They found that while red edge features changed with seasonal stage of development, there was a sustained period in which values in deciduous trees tended generally to longer wavelengths, between Julian dates 156-222 (about June 5 until August 10). This 9-week period could possibly be used as a sampling window for red edge features that would be minimally influenced by season.

Also using stacked layers of *Eucalyptus* leaves, Datt (1999) reported a new reflectance index for remote sensing of chlorophyll content: (R850-R710)/(R850-R680), which was more sensitive than a range of other indices, and presumably less sensitive to scattering. Simple ratios of reflectances at 2 wavelengths (e.g. R750/R700 and R750/R550) provide a first order approximation of biochemical content only if the leaf surface reflectance is negligible.

Leaf age

We found that young leaves (e.g., *A. saccharum*) or aging leaves in which chlorophyll breakdown had begun (e.g., 1-year-old foliage of *Pinus strobus*) produced higher reflectance in certain regions of the VIS spectrum. The region most affected depended on the species, e.g., young *A. saccharum* foliage was red in colour, hence, showed an increased red reflectance. In *Pinus strobus* and *Tsuga canadensis*, aging or young

foliage were lighter green than current-year mature or near-mature foliage, and consequently, reflectance was higher in the green bands. These foliar age effects were also evident in spectral index values, particularly PRI.

The implication of these findings for scaling up spectral reflectance to the remote scale is that spectral results should be affected by the relative proportions of young and old foliage in a canopy, and these effects will vary depending on the species. This will need to be considered when drawing conclusions from canopy-level spectra.

In other work, Roberts et al. (1998) found that older leaves of 6 Amazon species had decreased reflectance and transmittance and increased absorptance; NIR changes were most significant. During leaf expansion, leaf water content and specific leaf area decreased rapidly. Over the first 6 months, spectral changes occurred across the spectrum, resulting in decreased transmittance and increased absorbance in the VIS and NIR ranges, and decreased visible and increased NIR reflectance. Changes were detectable with remote sensing and could be used to map the species caatinga and monitor interannual or seasonal variability in phenology. They concluded that if these results can be extended to other communities with long-lived foliage, they may offer a means for mapping vegetation on the basis of leaf longevity.

Species differences

Blue reflectance was found to be particularly high in certain species, notably *Abies balsamea*, lichen and certain grasses. [Lichen has been reported to be highly reflective (Leckie et al. 1989) in remotely sensed images and its presence on the branches of defoliated trees will greater alter branch reflectance characteristics.] Some distinction between *Picea* and *Pinus* in the blue wavebands was also noted which was consistent with the earlier findings of Middleton et al. (1998) that blue adaxial reflectance could separate boreal tree species (*Pinus banksiana, Picea mariana,* and *Populus tremuloides*), even considering seasonal changes.

While the 44 species investigated here displayed some segregation of spectral profiles according to functional type, particularly in the blue and NIR wavebands, spectra were frequently quite similar among species. Within a species, certain factors, e.g., leaf stage of development, leaf age, leaf surface, and stress effects, had pronounced effects on spectral characteristics.

Despite the mixed findings for species discrimination at the leaf level, we cannot conclude that spectral features will not be useful in identifying species at the canopy scale, since canopy assessments can also take into account species-specific tree architectural effects. Further, advanced analytical techniques such as neural networks may be able to distinguish subtle spectral details (Gong et al. 1997). Nonetheless, Price (1994) wisely suggested that spectral libraries should include a number of examples for each species to provide an adequate description of within-species variability.

Stress effects

The types of stresses that plants are likely to encounter are manifold, and include insects, diseases, temperature extremes, drought, pollution, nutrient deficiencies or toxicities, vegetative competition, and mechanical damage. Spectral information cannot be expected to discern among stresses because (1) stresses often act in concert and (2) many of these stresses produce similar physiological effects. Hence, reflectance should not be used to diagnose the

specific cause of stress. Secondly, changes in reflectance characteristics do not always denote a stress problem, as physiological features undergo a certain amount of non-stress related variation and transient non-damaging strain, diurnally and/or seasonally.

Ratio-based indices are commonly used to measure sensitivity of vegetation to stress (Dendron Resource Surveys 1997). These indices typically combine reflectance from stress sensitive and insensitive bands. For example, Gitelson and Merzlyak (1996) found a high correlation between R700/R750 and R550/R750 and the chlorophyll content of maple and chestnut (*Castanea* spp.) trees.

The reflectance region most studied and with the most sensitivity to stress-induced change is the red edge, which Horler et al. (1983) defined as "the rise of reflectance at the boundary between the chlorophyll absorption feature in red wavelengths and leaf scattering in near infrared wavelengths" (Dendron Resource Surveys 1997). The position of the red edge is consistent among different species and generally ranges from 680 to 750 nm. Stress on vegetation has been shown to cause a shift of the red edge inflection point, REIP, to shorter wavelengths, a so-called *red edge – blue shift*.

Vogelmann et al. (1993) showed such a shift in the laboratory with declining chlorophyll content from insect damage in *Acer saccharum* leaves. Boyer et al. (1988) studied senescence and spectral reflectance in leaf stacks of *Quercus palustris* and found that the Gaussian model of the red edge described by Hare et al. (1984 a, b) and Miller et al. (1985), provided a reliable way of discriminating among 6 stages of senescence. Miller et al. (1991) studied red edge reflectance in 10 species (using stacked leaves), including *Acer saccharum*, and suggested that red edge position could possibly serve as an indicator of the presence or absence of stress rather than a means of measuring degree of plant stress. In applications at the remote scale, Rock et al. (1988), found that forest decline in *Picea* and *Abies* was associated with a 5-nm blue shift in the REIP.

Others have observed some general patterns of spectral response. Leckie et al. (1989) looked at spectral characteristics of tree components of *Abies balsamea* and *Picea* damaged by spruce budworm (*Choristoneura fumifera* Clem.) They found that current-year needles had significantly higher reflectance than older needles in the green and yellow parts of the spectrum. Wilson et al. (1998) investigated the spectral reflectance characteristics of Dutch elm disease and found an associated drop in NIR reflectance, consistent with findings of other studies of forest damage, and an increase in green and red reflectance. Luther (1999) found that foliar spectral reflectance from stacked leaves could be used to develop an index of *A. balsamea* vigour. Reflectance decreased consistently with vigour throughout the spectral region analyzed (350 to 2500 nm).

Leaf water content is generally considered to be the primary factor affecting leaf reflectance in the infrared wavelengths from roughly 1300 to 2500 nm (Treitz and Howarth 1999). Carter (1991) found that water content influences the spectra across the 400 to 2500 nm region, with the greatest effects in the water absorption bands near 1450, 1940, and 2500 nm; sensitivity maxima were also located between 400 and 720 nm. As an indicator of plant strain, however, leaf water content is less sensitive than leaf chlorophyll content, appearing only at advanced stages of leaf dehydration (Carter 1993). In a study of reflectance and fluorescence spectra, Philpot et al. (1996) found that reflectance from single leaves from soybean plants subjected to water stress differed little from reflectance of the leaves from

control plants. However, blue and red fluorescence was somewhat greater for the stressed plants. Unusual fluorescence features in the yellow-green part of the spectrum were also found in both control and stressed plants; these features were exceptionally sensitive to stress.

Another index, NDVI, has been widely reported in the literature, and has been useful mainly at remote scales within certain leaf area index thresholds (e.g., Gamon et al. 1995a). In our study, NDVI-based indices were indicators of early strain in *P. banksiana* but not the other conifers examined. In other leaf-level studies, Vogelmann et al. (1993) found NDVI did not correlate with total chlorophyll as well as REIP. The 3 red edge parameters were apparently less influenced by differences in green leaf biomass and background condition than NDVI. Luther (1999) found that the normalized difference vegetation index (using 711 nm as the most significant band and 913 nm as the insignificant band) correlated strongly with concentrations of chlorophyll *a*, chlorophyll *b*, nitrogen, and shoot lengths thereby comprising a good integrative index of *A. balsamea* vigour. There were also significant regions between 1310 to 1500 nm, but under field conditions this region was limited by atmospheric absorption.

The Photochemical Reflectance Index appears to be worthy of further investigation. When treatments and species were statistically pooled in the herbicide and girdling stress experiment to provide a heterogenous mixture of 40 sample means, a correlation coefficient of 0.61 was found between PRI and subsequent growth and browning in the pooled data set (Table 15). The implication is that indices such as PRI may serve as non-specific indicators of early strain in plants, but this needs further study.

The advantage of non-specific indicators is their ability to bypass the confounding influence of species and target physiological strain. Gamon et al. (1995b) suggested that PRI derived from top canopy leaves was relatively unaffected by species or functional type. However, they cautioned that at scales larger than the single leaf, this signal can be confounded by varying pigment composition and the presence of non-green material such as soil, complicating an unambiguous physiological interpretation of this index. They proposed an alternative approach for remote assessments by deriving physiological information from residual spectra after fitting fundamental spectral components in the image.

Conclusions

The indices used in this study are examples of a rapidly expanding number of spectral indices reported for use in near-field, airborne, or satellite remote sensing. However, many indices have not been extensively tested. Testing is essential for developing practical applications for hyperspectral methods in forestry.

Leaf reflectance was able to provide some broad distinction of species groups, but accurate identification of species at the leaf level is difficult owing to the considerable degree of intra-species variation in reflectance characteristics. Factors such as leaf age and stress effects can easily supercede species effects. However, the capacity to distinguish species may be more successful at the remote canopy level, taking into account species-specific tree architectural effects.

Scaling from leaf to canopy is a complex process and introduces many additional structural, physical, and biological elements. Asner (1998) concluded that the structural

attributes of ecosystems determine the relative contribution of tissue, canopy, and landscape factors that drive variation in reflectance signal. Based on experimental and modelling evidence, vegetation reflectance is known to be primarily a function of tissue optical properties, canopy biophysical attributes, soil reflectance, illumination conditions, and viewing geometry (Treitz and Howarth 1996).

The use of spectral properties in the early detection of physiological strain appears promising. Here, several newer spectral indices showed promise, and could be integral components of a monitoring program for detecting changes in forest health. These indices, and others, are being explored further at the remote scale in our related research on the development of bioindicators of physiological condition (Zarco-Tejada et al. *a, b*; Mohammed et al. 1997).

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